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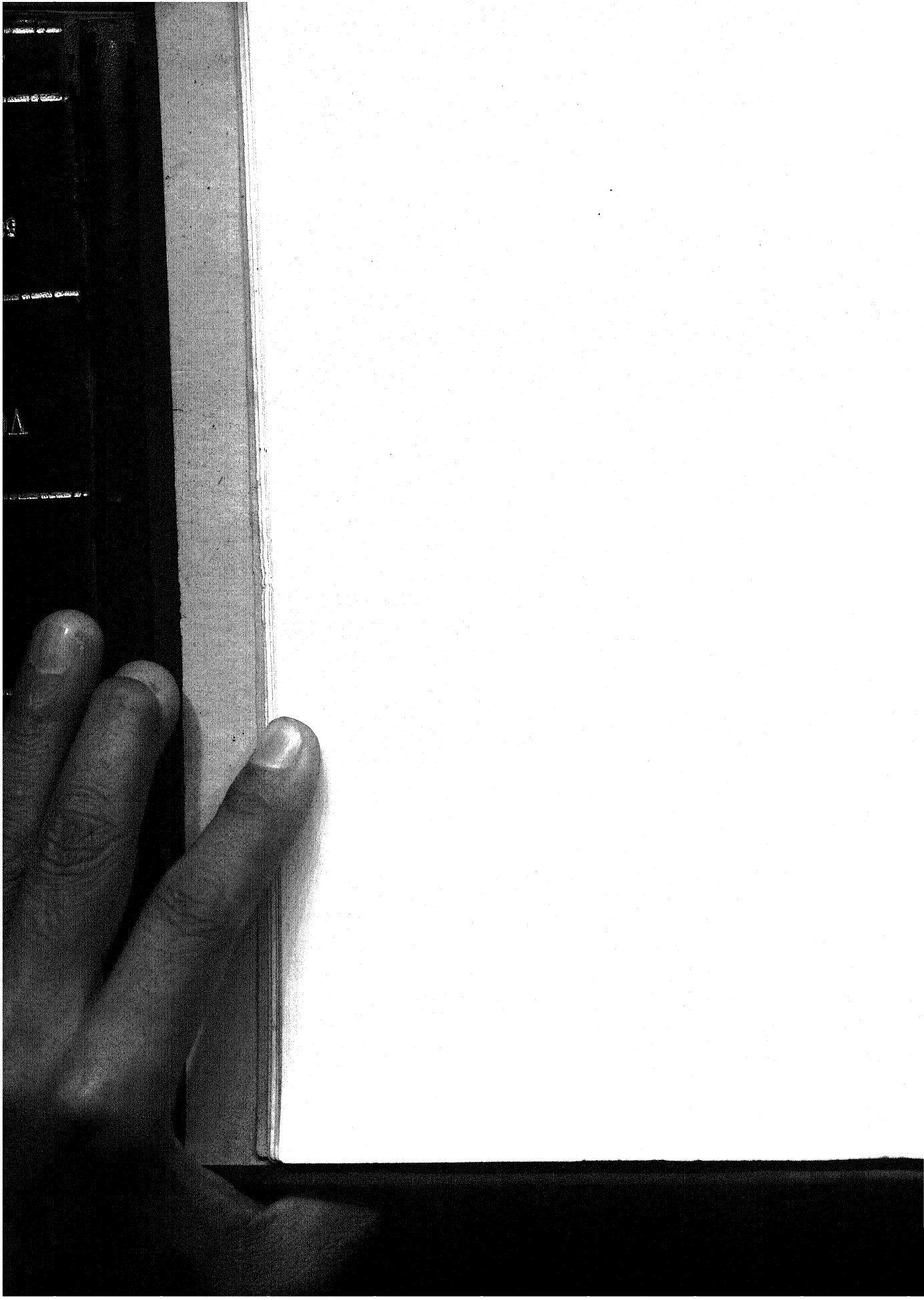
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ERRATA, VOLUME XVIII

Page 139, 5th line from top of legend. For *carcasana*, read *caracasana*.
Page 176, 5th line from top. For *Bauhinea*, read *Bauhinia*.
Page 353, 7th line from end of text. For *laterita*, read *lateritia*.
Page 754, 6th line from top. For *caracasana*, read *caracassana*.



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TWO SPECIES OF *VALSA* CAUSING DISEASE IN *POPULUS*

ERNST J. SCHREINER

(Received for publication June 11, 1930)

INTRODUCTION

Similar canker diseases on poplars are caused by two species of fungi of the genus *Valsa* Fries. One of these is *Valsa sordida* Nitschke (1867), the pycnidial stage of which has been known as *Cytospora chrysosperma* (Pers.) Fries (1823). This pathogen, though often merely saprophytic, is the causal agent of the canker disease of poplars first described by Long in 1918 as due to the fungus *Cytospora chrysosperma*. The perfect and imperfect fruiting stages of this fungus often grow together on the same stem, and taxonomists (such as Diedicke, 1912; Grove, 1923; and others) have claimed that the two stages are of the same fungus, but apparently the relationship was not verified by culture experiments. Single ascospore cultures of *Valsa sordida*, isolated in the course of this work as described below, have always produced the pycnidial stage known as *Cytospora chrysosperma*, both on agar media and on sterilized twigs, thus proving the two fruiting forms to be stages of the same fungus. The ascosporic stage was never developed in any of the cultures.

During my investigations it was found that another species, *Valsa nivea* (Hoff.) Fries (1849), not previously reported as parasitic, occasionally causes cankers on poplars very similar to those caused by *V. sordida*. The pycnidial stage of this fungus has been known as *Cytospora nivea* (Hoff.) Sacc. (1881). The relationship of the two spore stages, which were associated by taxonomists but apparently never proven by culture methods to be the same, has also been established from single ascospore cultures during the course of the work, though here again the ascosporic stage never developed in culture. The diseases caused by these two species of *Valsa* are so similar in appearance that unless the fruiting bodies are present it is impossible to determine which fungus is involved.

TAXONOMY OF THE GENUS VALSA FRIES

The generic name *Valsa* was applied by Fries in 1849 to certain of the species in the large group of the family Sphaeriaceae which up to that time

had been included in the genus *Sphaeria*. Fries described the genus *Valsa* as follows: "Perithecia carbonaceae (forma definitae), perfecta, circinatia, in colla convergentia elongata, ostiolis erumpentibus inter se vel disco conjunctis. Asci (typice) perfecti, 8-spori."

One of the first attempts at a thorough systematic work on the Pyrenomycetes was made by Nitschke in 1867–1870. He separated families chiefly on the basis of the position and structure of the stroma and of the perithecia. The general characters of the spores (color, shape, etc.) were used in separating genera. Nitschke divided the genus *Valsa* into 5 subgenera and included 102 species. Later writers such as Winter (1887) and Lindau (1897) accepted Nitschke's classification. In the genus *Valsa* Winter listed nine subgenera with 128 species and Lindau listed 10 subgenera.

Saccardo (1882) based the major divisions of the family Sphaeriaceae on the character of the spores. He retained the genus *Valsa* Fries only in part, limiting it to the subgenera Euvalsa and Leucostoma of Nitschke. The essential characteristics of the genus *Valsa* as described by Saccardo are as follows: Spores allantoid, hyaline or olivaceous; perithecia embedded in the bark, more or less circinate and in a single layer; ostioles entire and converging, usually united in a small erumpent disk; the stroma may be indistinct without any circumscribing line, disk usually black (Euvalsa); or it may be well developed and circumscribed by a black line, disk usually white, cinereous, or yellowish (Leucostoma). Ellis and Everhart (1892) have followed Saccardo in naming the American species which they collected.

Ruhland (1900) came to the conclusion that the structure of the stroma, although varying in the group as a whole, is constant and characteristic for the individual species (p. 75). Ruhland used the term "ectostroma" to designate the stromatic mass which develops between the bark parenchyma and the periderm, usually composed only of fungus hyphae. He applied the term "entostroma" to the deeper stromatic mass which develops in the bark parenchyma and usually contains portions of the dead bark tissue. Although Ruhland pointed out that in the Euvalsa group there is a distinct ectostroma and a very diffuse, poorly developed entostroma in which the perithecia are formed, and that in the Leucostoma group the ectostroma is reduced and can hardly be differentiated from the very distinct and strongly developed entostroma, he did not definitely raise these groups to generic rank.

More recently von Höhnel (1917) has urged that a natural system of the Sphaeriales should be based primarily upon the character of the "nucleus of the perithecium." By this he referred to the arrangement of the asci in the perithecium, the characteristics of the asci and of the spores, and any other distinctive features within the perithecium. Von Höhnel raised the subgenera Euvalsa and Leucostoma, included by Saccardo in *Valsa*,

to generic rank, apparently mainly on the basis of the characters of the stroma, and kept the genus name *Valsa* Fries p.p. for the species in the subgenus Euvalsa. The stroma in *Valsa* as limited by von Höhnel is poorly developed and hardly distinguishable from the bark tissue, without any dark marginal line. The disk is seldom pure white. The stroma in *Leucostoma* (Nitschke) von Höhnel is well developed and limited by a distinct dark marginal line or zone. The disk is often pure white. Wehmeyer (1926), writing on the phylogeny of the stromatic Sphaeriales, agrees with von Höhnel on the value of the nucleus of the perithecium in determining phylogenetic relationships in this group, and on the separation of *Valsa* and *Leucostoma* as distinct genera. There is some justification on the basis of the characters of the stromata, already noted above, for the separation of *Valsa* (Euvalsa) and *Leucostoma* as two distinct genera. On the other hand, the similarity of the asci and spores of the species in these two groups would seem to make their retention in the genus *Valsa* more advisable, at least until more definite data on the life histories of the species concerned are at hand.

The species of the genus *Valsa* as limited by Saccardo are widely distributed and generally saprophytic, and many can undoubtedly grow on a wide range of substrata. The distinctions between described species are sometimes very slight, and it seems probable that in such cases the differences are due to variations of the same species under different environmental conditions. With our present limited knowledge of the biology of this group it is difficult to determine whether a given specimen is within the limit of variation of the particular species which it most resembles.

Description of the Fungi and of the Disease

Valsa sordida Nitschke

Both the perfect (*Valsa*) and the imperfect (*Cytospora*) spore stages of *Valsa sordida* have been known for over a century, but they have usually been considered as of secondary occurrence on dead branches. Long (1918) described this fungus, of which he found only the pycnidial stage (*Cytospora chrysosperma*), as a wound parasite on eleven kinds of poplar and three species of willow. He stated that *Populus Wislizenii* is highly resistant to the disease when given good care. Hubert (1920) reported *Cytospora chrysosperma* as a wound parasite on poorly growing trees of four kinds of poplars, three species of *Acer*, two of *Salix* and one each of *Prunus*, *Sambucus*, and *Sorbus*. Povah (1921) found the disease in New York State on young trees of *Populus grandidentata* and *P. tremuloides* weakened by fire injury, and Moss (1922) reported the disease on three kinds of poplars, and on *Acer saccharinum* in Ontario. The so-called Cytospora canker disease has also been described in numerous publications of a popular nature by state and federal experiment stations.

Togashi (1924) notes the disease caused by *V. sordida* as common on

Lombardy poplars in the vicinity of Hokkaido University, Japan. Evans (1922, p. 57) mentions it as occurring on poplar in South Africa. *Valsa sordida* and its pycnidial form *Cytospora chrysosperma* have often been collected in Europe, and Grove (1923) notes that this *Cytospora* causes a serious disease of poplars in England. Day (1924), in his description of the bacterial "Watermark disease" of the cricketbat willow in England, states that *Cytospora chrysosperma* is sometimes of secondary occurrence on trees weakened by the disease.

The fungus develops saprophytically on dead branches. Sucker shoots and branches girdled by insects often become infected, and pycnidia are readily formed on such a substratum. Numerous pycnidial stromata appearing as convex protrusions develop immediately under the epidermis and finally break through it, exposing a grey to black disk, in the center of which a single pore soon appears (Pl. I, fig. 10). During damp weather the pycnospores are pushed out through this opening in coiled, orange-colored spore horns, as much as 3/4 to 1 inch long. When there is an excess of moisture the spores exude in globular masses which soon spread out or are washed away.

The labyrinthiform pycnidial chambers (loculi), opening into a common central pore or into adjacent chambers, are embedded in a very diffuse stroma, without any distinct black, stromatic layer (conceptacle) around them (Pl. I, fig. 11). Pycnidial stromata produced under natural conditions usually showed a distinct greyish ectostroma and a darker-colored but more diffuse entostroma in which the pycnidia developed.

Occasional stromata did not show any clear differentiation into ectostroma and entostroma, a condition which was also often observed in pycnidial stromata developed in the bark of twigs in culture tubes. Pycnidia and spores were frequently developed in both the apparent ectostroma (*i.e.* ectostroma judging from position) and entostroma under cultural conditions, and in such cases the mature stroma appeared uniform throughout. Further work is necessary to determine the nature and relationship of the ectostroma and entostroma of Ruhland in this species.

All over the inside walls of the chambers, short filiform hyphae, the sporophores from which the pycnospores develop, project out into the pycnidial chambers. The loculi are usually filled with loose spores which are pushed out of the ostiole when the fruiting body becomes wet. The pycnospores are allantoid and hyaline, and vary in size from 3.7–5.4 × 0.8–1.1 μ, averaging 4.5 × 0.93 μ.

The perithecial stage also develops on dead stems but appears to be comparatively rare, at least in this country. Grove's discussion (1923, p. 4) seems to indicate that this stage is rather common in England, but Long (1918) reports finding only the pycnidial stage in the United States. Povah (1921) found *Valsa sordida* associated with *Cytospora chrysosperma* on diseased poplars at Syracuse, N. Y. The writer has

made collections from diseased poplars during all seasons of the year since 1924, but the perithecial stage of *Valsa sordida* was not observed until the fall of 1929. During the fall and winter of 1929–30 the perithecial stage has been rather abundant on dead branches of the poplars at The New York Botanical Garden, and it has been found on branches as small as 1/4 inch in diameter. The perithecial stage was also found on dead twigs of *Populus Maximowiczii* collected in Oxford County, Maine, and on dead branches of *P. tremuloides* collected near Johnsonburg, Pa., during the fall of 1929. The perithecia are developed deeper in the bark than the pycnidia, in a very diffuse and indistinct stroma which does not have any line of demarcation around the edge of the stromatic tissue. There is so little stroma that the dead bark tissue can easily be teased away from the groups of perithecia (Pl. I, fig. 8). The ostioles of the perithecia are usually arranged more or less regularly in a circle around the edge of a blackened or dirty-grey disk (Pl. I, fig. 6). Occasionally the central disk is lacking and the ostioles are then grouped in a compact mass (Pl. I, fig. 7). The central disk appears to be the remnant of an old pycnidium, since pycnospores have always been found in this mass of broken down fungus hyphae in considerable quantities. A section through a perithecial stroma shows no ectostroma which can definitely be distinguished from the greyish mass containing pycnospores. Whether this greyish mass is actually an old pycnidium or the perithecial ectostroma could not be definitely determined from the mature material at hand. To settle this important point, it will be necessary to study the origin and development of the perithecial stroma in all its stages.

The perithecia are flask-shaped, black, and rather thin-walled, and vary in size (Pl. I, fig. 9). The average diameter of the body of the perithecia is roughly 0.5 mm., varying from slightly less than 0.4 mm. to over 0.74 mm The necks of the perithecia average between 0.5 and 0.6 mm. in length the shortest measured was less than 0.4 mm. and the longest was over 0.75 mm. The neck is slender in proportion to the size of the body, and it is always somewhat thickened at the ostiolar end. The ostioles of the perithecia are "entire," very slightly or not at all erumpent and the ostiolar walls are firmly attached to each other and to the epidermis. The epidermis splits open in a rather irregular manner, but the outline of the exposed portion of the fruiting body is usually more or less circular and varies from 0.5 to 1.0 mm. in diameter. The number of perithecia in a group may vary from 2 or 3 to as many as 16 or 18.

The asci are narrow, club-shaped, sessile or very short stipitate, and 8-spored. Their size (p.sp.) varies from 26–43 × 4.3–6.4 μ, the average size being 33 × 5.2 μ. Including the stipe-like base the average size is 40 × 5.2 μ. The ascospores are hyaline and allantoid. The average spore size in our specimens is 9 × 1.5 μ, ranging from 6.6–10.9 × 1.3–1.7 μ.

This *Valsa* belongs in the subgenus Euvalsa Nitschke, the species of

which are characterized by the lack of a definite stroma, and by the asci with short, evanescent stalks and hyaline spores. Our specimens agree with the descriptions of European collections of *V. sordida* in all but the ascus and spore size which are given as 48–60 × 8 μ, and 12 × 1.5–2 μ, respectively. The average size of the asci in our material is 40 × 5.2 μ, and the average size of the spores is 9 × 1.5 μ. This is not a sufficient difference to justify the assumption that our specimens belong with a different species, or even with a variety of *V. sordida*. Our material agrees in spore size with those reported by Ellis and Everhart (1892) in their description of *V. sordida* from Iowa, which they give as 8–10 × 1.5 μ. Such differences in size may be due to various factors. The spores of *Valsa* swell rapidly previous to germination, and under certain moisture conditions the spores swell in the perithecium itself. Rolfs (1910), working with *V. leucostoma* on peach, found that the average size of the spores was 8–14 μ × 2.5 μ. Often the spores became so swollen in the perithecium that when they were finally pushed out they measured 12–15 × 3–5 μ. Further, there may be geographic strains of the same species which vary in spore size. Until more is known as to the range of variation within a species of this group of ascomycetes, our specimens must be considered as *V. sordida*.

Valsa sordida is a facultative parasite, and the severity of the injury to the tree, *i.e.* the degree of parasitism of the fungus, is usually greatest on poorly growing trees or branches. This fungus has been found on all kinds of poplars in New England and New York. It is commonly parasitic on the native aspens *Populus tremuloides* and *P. grandidentata*, and it often causes serious damage to the Lombardy poplar. Dormant cuttings stored in moist sand are especially susceptible to severe infection, sometimes with a mortality of 90–100 percent, when the temperature is not kept below 2° C. to prevent the growth of the fungus.

On living branches the fungus enters through wounds or dead twigs, kills the cambium, and spreads rapidly in the bark. If the branch is killed quickly by a rapid spread of the mycelium the dead bark becomes loosened and begins to slough off. Where the growth of the fungus is slower, the dead bark becomes depressed and often develops longitudinal cracks as it dries. The continued growth of the adjacent healthy bark and wood makes this sunken area, the so-called canker, which is usually more or less spindle-shaped or narrowly elliptical, more pronounced (Pl. II). Pycnidia often develop on the sunken area of even comparatively small cankers.

The mycelium of *V. sordida* ramifies throughout the dead bark tissue, within the walls of the more or less crushed and partially disintegrated cells as well as between them. The older hyphae are brown and sometimes form small dense mats. In most cases there is a sharp line along the edge of the canker without any transition zone between dead and living cells, and the hyphae were not found penetrating into the living cells or growing

between them. Occasionally on the edge of cankers which are enlarging rapidly there is a very narrow zone in which a few hyphae may be seen among cells which are apparently not entirely dead. The wood is stained brown and the mycelium is found in the vessels, the fibers, to some extent in the wood parenchyma, and also between the medullary ray cells, but they apparently penetrate into the latter only when the cells are dead. On small stems the mycelium is also present in the pith which is stained the same color as the wood.

The growth of *V. sordida* in the bark and wood of poplar is similar to the growth of *V. japonica* in *Prunus* as described by Hemmi (1916), and the growth of *Endothia parasitica* in chestnut as described by Keefer (1914). Keefer's observations indicate that the injury to the living cells may be mainly of a mechanical nature, due to the growth of the mycelial weft, possibly aided to some extent by enzym action. *Valsa sordida* is much less parasitic than *Endothia parasitica*, although the growth of the former as a saprophyte seems to be more vigorous than that of the latter. It seems probable that in the case of *V. sordida* enzym action precedes the advance of the mycelium, as shown for *Botrytis cinerea* by Brown (1915), and Blackman and Welsford (1916), and for *Sclerotinia Libertiana* by Boyle (1921).

If the growth of the host limb is vigorous, the living tissue on each side of a small canker may approach and cover the infected tissue in a year or two. The fungus is then in effect sealed in, but it may remain alive in the wood for a number of years (Pl. V, fig. 6). Where the host branch is less vigorous the fungus frequently kills the living bark around the canker at about the same rate as the growth of this tissue, and a rather deep hole or scar, often the shape of the original canker, results after a year or two. Many of these slowly growing cankers appear like insect burls (Pl. V, figs. 1, 2). On limbs of still less vigor the fungus may develop more rapidly, forming a progressively longer and wider canker which finally girdles the entire limb. In some cases the cambium behind the roll-like boundary of wound tissue is killed by the fungus. A badly infected tree may present all of these types of cankers, and even the same canker may assume all the different phases described at various seasons of the year or in successive years. The development of this disease is much the same as that caused by *Valsa leucostoma* on apple, cherry, etc., as described by various writers, and similar to the disease caused by *Nummularia discreta* (Schw.) Tul. on apple, as described by Glover (1921).

Cankers on large branches and trunks usually start around the base of dead stubs or smaller branches which have been killed by the fungus. McCubbin (1918) notes that this is also true in the case of the peach disease caused by *Valsa leucostoma*, and both Togashi and Hemmi in their studies on *V. Mali* and *V. japonica* found that the percentage of infection was higher, and the resulting injury to the living branch was much more severe,

when they inoculated on burned areas rather than on clean knife cuts. The small patches of dead tissue seemed to give the fungus a more vigorous start. Although infection of a large branch or trunk by *V. sordida* is often through a smaller infected branch, this is not necessarily always the case. The fungus may enter through any wound in the bark and if the wound is deep enough to kill the cambium the course of the disease is usually as described above. If a shallow bark wound in which the cambium is not killed becomes infected during the growing season, the infected area is often cut off from the healthy tissue by a more or less definite abscission layer. When such an infection takes place during the dormant season or on a very weakly growing branch or trunk, the fungus may kill the cambium and develop a typical canker.

Valsa nivea (Hoff.) Fries

Valsa nivea (Hoff.) Fries has only occasionally been found by the writer as the obvious causal agent of cankers on living poplar trees. This fungus usually forms slowly growing cankers, which appear exactly like those caused by *Valsa sordida* on the more vigorous trees, but a few cases of large rapidly growing cankers have been observed. From our observations to date *Valsa nivea* does not appear to be as virulent a parasite as *Valsa sordida*.

It is impossible to distinguish a canker caused by this fungus from one caused by *V. sordida* unless the fruiting bodies are present. The pycnidia of *Valsa nivea*, which also develop on dead twigs and branches, can usually be distinguished from those of *V. sordida* by their dark red spore horns, and the white stromatic disk exposed by the ruptured epidermis (Pl. I, fig. 4). This last character is apparently variable, and the disk in some cases is dark grey to almost black. There is a single black pore in the center of the disk.

The pycnidial stroma is well developed, and has a well defined black zone of demarcation known as the conceptacle. The pycnidial stromata are uniform throughout, without any apparent differentiation into ectostroma and entostroma (Pl. I, fig. 5). The labyrinthiform pycnidial chambers, lined with short filiform sporophores, open into the central pore. The pycnospores usually fill the entire cavity, and are pushed out in long, coiled spore horns when sufficient moisture is present. The spores are allantoid and hyaline, and in our specimens measure 4.3–5.9 × 0.8–1.1 μ, averaging 5 × 1 μ. This is smaller than the spore size reported for European specimens, which is given by Saccardo (1881) and others as 6–6.5 μ × 1.5–2 μ.

The perithecial stage of *Valsa nivea* has been collected by the writer only once, on dead branches of *P. tremuloides* in Nova Scotia, in the fall of 1929. Judging from this material the description of the perfect stage is as follows: The black, flask-shaped perithecia are smaller than those of

V. sordida and the necks are usually somewhat thicker in proportion to the size of the body (Pl. I, fig. 3). The diameter of the body varies from about 0.25 mm. to 0.45 mm., averaging between 0.3 and 0.4 mm., and the length of the neck varies from 0.4 to 0.6 mm. The neck is thickened at the ostiole, which is "entire" and very slightly or not at all erumpent, and opens in a small pore.

The perithecia are embedded in a well defined, broadly flask-shaped, black stroma, which is rounded at the base. The perithecial stroma also appears uniform without differentiation into ecto- and entostroma. Ruhland (p. 46) working with this species also notes this fact. The stroma to which Nitschke applied the term "conceptaculum," is composed entirely of fungus hyphae; there are no remnants of the bark tissue included within it (Pl. I, fig. 2). It is rather brittle, and when it is broken open the perithecia can be seen embedded in a greyish white mass of fungus tissue. If the dead bark tissue is teased away from the stroma it appears as a hemispherical black mass. The perithecia vary from 6–18 or even 20 in a stroma. The epidermis is split open irregularly and becomes attached to the stroma around the edge of the exposed disk. The diameter of the disk varies from 0.5 mm. to 1.0 mm., averaging between 0.5 and 0.75 mm. The disk in our specimen is only seldom distinguishable because the ostioles are grouped so close together (Pl. I, fig. 1). Where the ostioles are more scattered they appear as small black dots on the greyish white disk. No stroma with a pure white disk was found on our material.

The asci are clavate, subsessile, and 8-spored, and their average size is $30 \times 5\ \mu$ (ranging from $23–38 \times 4.3–5.3\ \mu$). The spores are hyaline and allantoid, and vary in size from $7.0–9.1 \times 1.0–1.6\ \mu$, the average size being $8 \times 1.3\ \mu$.

This form fits in Nitschke's subgenus Leucostoma, which is characterized by the strongly developed, black stroma, broadly flask-shaped and rounded at the base. In this subgenus only one species, *Valsa nivea* (Hoff.) Fries, has been reported on poplar. Our specimen conforms to the descriptions of this species fairly well, although the snow-white disk with the rather scattered black ostioles breaking through it, which is described as characteristic of this species, is lacking in our specimens. Stromata with a greyish white disk and scattered black ostioles can be found here and there, but they are comparatively rare. The snow-white disk is probably not a constant characteristic. Leonian (1921) found that the plum strain of *Valsa leucostoma*, which normally has a white disk, did not show this characteristic when grown on apple branches. He also states that although the apple strain of *V. leucostoma* does not develop a white disk in the New Mexico climate, such a disk is formed in culture tubes under properly controlled moisture conditions. Our specimen also differs from *V. nivea* as described from European specimens in its spore size (given as $12–14 \times 3\ \mu$ by European writers), but agrees with the spore size given by Ellis and

Everhart (1892) in their description of specimens from Colorado and Montana, 7–9 $\mu \times 1.25$–$1.5\ \mu$. In spite of these variations from *Valsa nivea* (Hoff.) Fries as described, in my opinion our specimen should be included in this species, at least until more is known of the limit of variation within this group.

Culture Work with Valsa sordida and V. nivea

Culture Media

The following culture media were used for the artificial culture of these two fungi.

1. Steamed poplar twigs: The stock cultures were grown on steamed poplar twigs in large culture tubes. In making these, about 2 cc. of 2 per-cent agar were placed in the bottom of a tube, a section of living twig was placed in each tube, and the tube was plugged with cotton. The tubes were sterilized in the autoclave at 15–20 lbs. for twenty minutes on two successive days, and then kept in a vertical position until the agar was solid.

2. Poplar decoction agar: A decoction was made by boiling twigs and buds of the Norway poplar in tap water for a period of four to five hours. One hundred cc. of this decoction and fifty grams of cane sugar were added to 18 grams of agar that had been dissolved in 900 cc. of tap water.

3. Bacto corn meal agar: Made by the Digestive Ferments Co., Detroit, Michigan.

4. Bacto potato dextrose agar: Made by the same firm as no. 3.

5. Synthetic nutrient agar: Ammonium nitrate, 10 gr.; dihydrogen potassium phosphate, 5 gr.; magnesium sulfate, 2.5 gr.; iron chlorid, trace; cane sugar, 50 gr.; agar agar, 15 gr.; distilled water, 1000 cc.

6. Steamed poplar leaves: Poplar leaves were sterilized in small flasks together with a small amount of water.

7. Poplar wood: Small blocks of seasoned poplar wood were boiled in tap water and then sterilized in culture tubes.

Method of Single Spore Isolation

All culture work (as well as the inoculation experiments) was carried out with single ascospore or single pycnospore cultures isolated in the following manner: Several drops of a spore dilution in sterile water were placed on the surface of poplar decoction agar in a petri dish and spread out as much as desired by tipping the dish in all directions. The small hyaline spores of *Valsa* were not visible with the low power of the microscope immediately after sowing, but in 24–36 hours, when the spores began to germinate, they could be easily seen. Single germinated spores were transferred with as little agar as possible, by means of a very fine needle, to another plate of the same kind of medium. The inoculum containing the single germinated spore was inspected under the high power immediately after transferring, and examinations of the isolations were then made at frequent

intervals. If any dormant spores which had been previously overlooked began to germinate, they were easily recognized, and the particular isolation was discarded.

When one or more hyphae had grown out of the original inoculum into the surrounding agar, the tips were cut off and transferred to another petri dish of the same kind of medium. None of the agar of the first transfer was carried over in this second operation. As soon as possible, hyphal tips were cut from this second culture and transferred to media in a culture tube, again leaving the agar from the previous transfer behind. This procedure was considered necessary in order that the small, hyaline, dormant spores (especially pycnospores), would not be carried over with the single germinated spore, possibly to germinate later in the culture tube.

Spore Germination

Ascospore Germination

The ascospores of *V. sordida* and *V. nivea* germinated readily on poplar decoction agar and on 5 percent sugar agar (agar 1 percent, cane sugar 5 percent). The germination of spores stored from one week to five months in an herbarium packet was observed on both kinds of agar. The ascospores of *V. sordida* germinated more quickly than those of *V. nivea*. The spores swelled previous to the formation of germ tubes, but they very seldom became spherical. They increased materially in diameter and slightly in length, but more or less kept their allantoid shape, at most becoming cylindrical. Germ tubes about 4 μ in diameter started from one or both ends in 12–15 hours after sowing, and they soon became septate, branched, and much bent. The spores of *V. japonica*, according to Hemmi (1916), germinate in an entirely similar way.

The ascospores of *V. nivea* swelled excessively, becoming egg-shaped to spherical, before the germ tubes developed. The swollen spores began to produce germ tubes about 7.5 μ in diameter from one side or from two diametrically opposite sides in about 30 hours. They formed septa early, but rather few branches, and normally they did not appear abruptly bent or convoluted. Rolfs (1910) describes much the same type of germination for the spores of *V. leucostoma*.

Ascospores of *V. sordida* and *V. nivea* were sown on decoction agar in the same petri dish to obtain germination under identical conditions. At the end of 24 hours at room temperature the spores of *V. nivea* had become considerably swollen, but no germ tubes had started (Pl. I, fig. 12), whereas the spores of *V. sordida* had already germinated and developed hyphae, occasionally over 200 μ in length, and 4–4.5 μ in diameter (Pl. I, fig. 14). After 46 hours most of the viable spores of *V. nivea* had developed germ tubes averaging 7.5 μ in diameter, and some had produced hyphae up to 250 μ long and 5 μ in diameter (Pl. I, fig. 13), while the germinated spores of *V. sordida* had produced an extensive and profusely branched mycelium (Pl. I, fig. 15).

Pycnospore Germination

The pycnospores germinated in the same manner as the ascospores. Germination of fresh pycnospores of both species on poplar decoction agar in the same petri dish is shown in Plate I, figs. 16–19. Twenty-four hours after sowing, the pycnospores of *V. sordida* were much swollen, averaging about 10.7 × 5.5 μ (those which were globose averaged 7 μ in diameter), and had begun to put out germ tubes, some of which were 20–30 μ long and 3 μ in diameter. The pycnospores of *V. nivea* were just beginning to swell. A very few spores had become spherical, but their diameter was seldom more than the original length of the pycnospore. At the end of forty-six hours the pycnospores of *V. sordida* had increased to an average size of 17 × 9 μ. A few were almost spherical, up to 8.5 μ in diameter, but most of them were very irregular in shape, and germ tubes in many cases seemed to start from any point on the spore. The primary mycelium was branched and septate, and the average diameter of the hyphae was 3.0 to 3.5 μ. The germinating pycnospores of *V. nivea* had all become greatly swollen and spherical, varying from 8–17 μ in diameter. Many had produced stout germ tubes, usually 8–8.5 μ in diameter, and some had developed hyphae over 250 μ long and 5–6.5 μ in diameter, which were often septate when 75–100 μ long.

Experimental Culture Work

The different appearance and rate of growth of the mycelium from single ascospores was noticeable in test tube cultures, and in order more fully to determine the extent of these differences, single spore cultures were grown in petri dishes under uniform conditions.

Since the mycelium derived from a single ascospore is propagated in culture by vegetative transfers, mycelium or pycnospores, such a culture may be called a clon, as noted by Stout (1929). Mycelial saltations, such as reported by Leonian (1926) in *Phytophthora*, or segregations in vegetative spores as reported by Dodge (1928, 1930) in *Neurospora tetrasperma* and in *Neurospora sitophila*, which give rise to a mycelium inherently different from the mycelium of the original single spore culture, must be recognized as new clons. Saltations did not occur in the single spore cultures of *V. sordida* and *V. nivea* during the course of this work.

For the comparison of mycelial characters and rate of growth, cultures were grown in 100 × 15 mm. petri dishes containing 25 cc. of media from the same flask. An equal volume of inoculum of the same age was assured by transferring disks 8 mm. in diameter, cut from a petri dish culture in a circle just within the outer edge of the mycelial growth. The disks were cut out with a small sterile vial, and were laid face down on the medium to be inoculated.

Types of Mycelial Growth on Poplar Decoction Agar

V. sordida. The mycelia developed from single ascospores obtained from perithecia on the same canker differed considerably when grown on

poplar decoction agar. The most noticeable differences were the regularity or irregularity of growth, the amount of surface and aërial mycelium, and the tendency to produce pycnidia. Such mycelial variations were shown by clons S-145 and S-155, both from ascospores of a specimen collected in Oxford County, Maine.

The mycelial growth of S-145 was very uniform, with an even, entire edge, little aërial mycelium, and a general greyish color (Pl. III, fig. 1). The growth of S-155 was somewhat slower than that of S-145, and it was much more irregular in appearance and more or less lobed at the edges. This clon also produced more aërial mycelium, and formed pycnidia earlier and in greater abundance than S-145. The young mycelium was white, but the older parts became brown to dark green (Pl. III, fig. 5). Other clons from the same source represented all gradations between these two distinct types.

Among the clons from a specimen collected near Johnsonburg, Pa., a slow-growing, lobed type of mycelial growth was observed. This type of growth was shown by clon S-176 (Pl. III, fig. 9). The mycelium tended to grow in rather distinct radial sectors, producing a very irregular margin. The white mycelium soon changed to brown or brownish green and pycnidia were produced comparatively early. The surface mycelium often formed a dense mat, and with the dense submerged mycelium this often produced a "heaped" effect along the edge of the mycelial mass. Clons with a regular growth habit (the S-145 type) were also present among the numerous single spore cultures from this source, and there were all variations between these two extremes. The type represented by S-155 was not found among clons from this source.

V. nivea. Two types of mycelial growth, with all intermediate gradations, were found among single ascospore cultures from the material collected in Nova Scotia. The first type (S-98) produced a rather loosely growing mycelium which was at first colorless, later becoming brown and then green. There was very little surface mycelium; the hyphae were mostly submerged and were usually difficult to see (Pl. III, fig. 13, lower mycelium). The second type (S-102) formed a white mycelial mass which was more dense and compact and had much more surface mycelium than that of S-98 (Pl. III, fig. 13, upper mycelium). The color soon changed to dark brown and finally to dark green in the older portions.

These mycelial characters of the individual clons of both species have remained constant on poplar decoction agar up to the present time (five months).

Description of Growth on the Various Media

The cultures from which the following data were obtained were all kept at a temperature of 25° C.

V. sordida. Growth on steamed poplar twigs: Growth was very rapid on this medium. The mycelium grew through the twig, forming white

cottony tufts at the upper cut end and at other points where the wood was exposed. As the growth continued, pycnidia and mycelium began to break through the bark, and after four or five weeks the twig was often overgrown with a dense mat of white or light grey mycelium, sometimes tinged here and there with light pink. Pycnidia were formed superficially as well as within the bark. The superficial pycnidia were globose and varied in size from less than 1 mm. to 5 mm. or more in diameter (Pl. IV, fig. 6).

Growth on poplar decoction agar: The rate of growth on this medium was somewhat slower than on steamed poplar twigs, and the habit of growth varied from very uniform to very irregular, depending upon the nature of the clon. The mycelium of clons with an irregular habit of growth and with a "heaped" appearance around the edges (type S-176) was usually very dense, whereas the mycelium of clons with a uniform habit of growth (type S-145) was of medium density. The color of the mycelial mass, which generally consisted of considerable surface mycelium but little aërial mycelium, varied from white to grey, changing to brown or green in the older portions and where pycnidia were forming. Clons of the S-155 type began to develop pycnidia after two weeks while others did not form pycnidia until 4 or 5 weeks after inoculation. The quantity of pycnidia produced also varied in the individual clons (Pl. III, figs. 1, 5, 9).

Growth on corn meal agar: The rate of growth on this agar was about twice that on poplar decoction agar. The clons which exhibited an irregular habit of growth on poplar decoction agar (S-155 and S-176 types) grew somewhat more regularly on this medium, but the irregular habit was still very much in evidence in the extreme types (S-176). The color was usually white, but some clons turned brown or green rather early. Pycnidia did not appear as early as on poplar decoction agar (Pl. III, figs. 2, 6, 10).

Growth on potato dextrose agar: The rate of growth on this medium was somewhat more rapid than on corn meal agar, and the growth habit of all clons was more uniform and regular. The clons which had a very irregular growth habit on poplar decoction agar were only very slightly irregular at the edges. There was much surface and aërial mycelium, forming a very dense mat, and the color was white with a tan or buff tinge. Pycnidia began to appear somewhat sooner than on the previous medium (Pl. III, figs. 3, 7, 11).

Growth on synthetic nutrient agar: Here the rate of growth of clons with a uniform habit of growth (S-145 type) was about a fourth as rapid as on poplar decoction agar, while that of clons with an irregular habit (S-176 type) was about half as rapid. Clons of all types developed an irregular mycelium which in all cases was very dense and had a decided "heaped" appearance at the edges. The color was pink with a slight tan tinge, in many cases turning dark green or brown on the older portions. Pycnidia had not developed on this medium up to six weeks after inoculation (Pl. III, figs. 4, 8, 12).

Growth on steamed poplar leaves: There was a great abundance of white surface and aërial mycelium giving the growth a cottony appearance. Pycnidia were formed on this medium in about 3 weeks.

Growth on poplar wood: The mycelium formed on poplar blocks was rather sparse, penetrating the block and forming a weft or thin mat over the surface. Small pycnidia were formed in 5 or 6 weeks on blocks which did not dry too much.

V. nivea. Growth on steamed poplar twigs: The growth of *V. nivea* on steamed poplar twigs was slower than that of *V. sordida.* As in *V. sordida,* the mycelium grew through the wood and pith of the twig and formed tufts at the upper cut end and at wounds in the bark. These tufts of mycelium were dark grey, almost black; in fact the whole twig had a blackened appearance. Many pycnidia developed in the bark, as they are normally found on natural infections. Globose, superficial pycnidia, varying up to 3 mm. in diameter and dark grey or black in color, were also formed on mats of surface mycelium (Pl. IV, fig. 11).

Growth on poplar decoction agar: The rate of growth on this agar was not as rapid as on steamed poplar twigs, and the character of the mycelium depended upon the nature of the individual clon. The mycelial mass was usually of medium density and varied in different clons from colorless or slightly brown to green or dark brown. Some clons produced considerable surface mycelium and some aërial mycelium, while others had little of the former and practically none of the latter.

Growth on corn meal agar: On this agar the average rate of growth was more rapid than on poplar decoction agar. Both the S-98 and the S-102 types appeared much alike, and produced a dense surface mycelium with some aërial mycelium. The color of the S-98 type was uniformly dark green to almost black, while that of the S-102 type remained white around the edges and was dark brown rather than green in older parts.

Growth on potato dextrose agar: The rate of growth here was about twice as rapid as on poplar decoction agar. The habit of growth and the color of the mycelial mass of the individual clons were the same as on corn meal agar.

The similarity of the cultural characteristics of the various clons on media where growth was very rapid, and also on media where growth was very slow, indicates a correlation between the cultural appearance and the inherent rate of growth. Apparently on a medium where growth is of medium rapidity (poplar decoction agar) the cultural characteristics of the clon are expressed to the best advantage.

Valsa sordida and *V. nivea* did not form perithecia on any of the media, either in single ascospore cultures or in mixed cultures. The production of perithecia in these forms may be dependent upon the proper environmental conditions, although there may also be heterothallism. Leonian (1923), by varying the culture media, could increase and decrease the

abundance of perithecia formed by one strain of *V. leucostoma*. He also found a strain of *V. leucostoma* which never produced perithecia under any cultural condition, and he concludes that this species has both a heterothallic and a homothallic strain. Wehmeyer (1924) obtained perithecia of *V. Kunzei* Fries in single spore cultures.

Rate of Growth on Poplar Decoction Agar at Various Temperatures

The rate of growth of various clons of both *V. sordida* and *V. nivea* under uniform conditions differed so much that it was decided to limit these studies to the average growth of the individual clons. The cultures were grown in uniform petri dishes on poplar decoction agar at 4° C., 9° C., 25° C., and 35° C., and the growth was measured at 24-hour intervals. From 4 to 8 cultures were used to obtain an average figure for the rate of growth at each temperature.

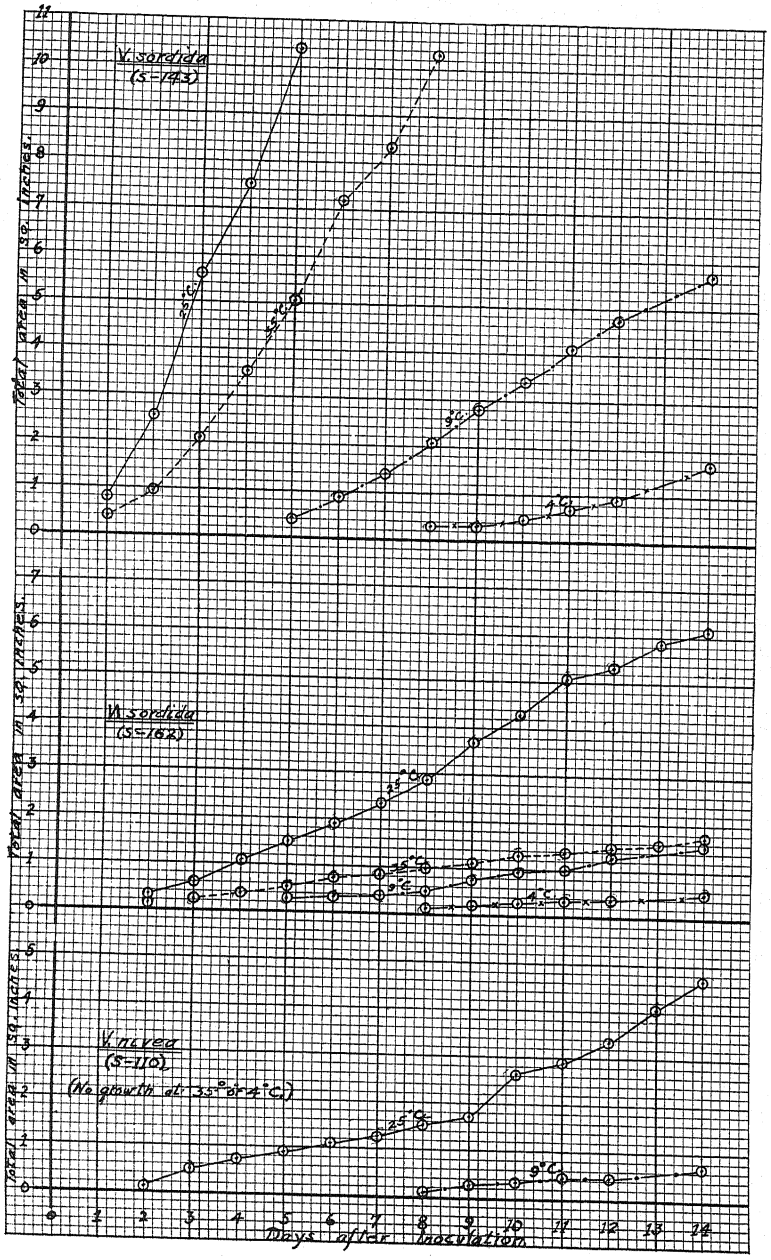
V. sordida. Two clons of *V. sordida* were used, one of which, S-143, was inherently fast growing and the other, S-162, was slow growing. The average growth of these clons is plotted in text figure 1. The growth of both clons was most rapid at 25° C., and less rapid at 35°, 9°, and 4° C., respectively. At 25° and 35° C. S-143 had grown a measurable amount 24 hours after inoculation. At 9° C. there was no measurable growth until the 5th day after inoculation, but the growth was then fairly rapid. At 4° C. there was no measurable growth until the 8th day and then growth was very slow, 1.65 square inches in 6 days.

There was no measurable growth on S-162 at 25° and 35° C. until the second day after inoculation. The growth of this clon at 25° C. was even slower than that of S-143 at 35° C. At 35° C. the growth of S-162 was very poor, less than that of S-143 at 9° C. At 9° C. S-162 showed no measurable growth until the fifth day, and at 4° C., none until the 8th day. The growth at both these temperatures was extremely slow.

Ascospore cultures were not kept at a temperature lower than 4° C., but in 1927 single pycnospore cultures of *V. sordida* from specimens collected at the New York Botanical Garden were kept at 2° C. They did not grow, and at the end of two weeks half the cultures were changed to 25° C. All the cultures at 25° C. started to grow within 2 or 3 days, but the cultures which were left at 2° C. remained dormant.

V. nivea. S-110 was taken as an average clon of this species. At 35° and 4° C. there was no growth during the entire period of 14 days. When half of the cultures were transferred to 25° C., they all started to grow within two or three days. At 25° C. there was no measurable growth until the second day. Growth at this temperature was only 4.75 sq. in. in 14 days, considerably slower than that of *V. sordida*. At 9° C. there was no measurable growth until the 8th day, and then growth was very slow, only slightly faster than the growth of S-162 at 4° C.

Although the rate of growth of different clons of *V. sordida* varies con-



TEXT FIG. 1. Growth of *Valsa sordida* and *V. nivea* at 4°, 9°, 25°, and 35° C.

siderably under the same conditions, all clons of this species grew at a temperature as low as 4° C. This would account in part for the severe damage by this fungus during the dormant season, which often looks like winter injury. The fungus can grow during the greater part of the fall, winter, and spring. In order to keep dormant cuttings in storage free from this disease, it is necessary to keep the temperature of the storage bins down to 2° C. The slow growth of *V. nivea* and its narrow temperature range undoubtedly influence its pathogenicity.

The characteristic differences in the rate of growth and in the appearance of the mycelium of clons derived from single ascospore cultures are evidence for the probable existence in nature of physiological strains of both of these species.

Growth on Steamed Twigs of Species Other than Poplars

Valsa sordida and *V. nivea* were inoculated on sterile twigs of 29 different species of plants representing 19 genera in the following eight families: Aceraceae, Betulaceae, Fagaceae, Magnoliaceae, Moraceae, Rosaceae, Ginkgoaceae, and Pinaceae. The living twigs were put into culture tubes which contained a small amount of plain agar (2 percent agar agar in water), and they were handled and sterilized in the same way as the steamed poplar twigs. The inoculum was placed on the hardened agar in the bottom of each tube.

V. nivea grew on twigs of all the species tested, and *V. sordida* grew on all except *Tsuga canadensis* Carr. In summarizing the results of these cultures, the growth of the two fungi on the various species of twigs has been compared with their respective growth on steamed poplar twigs. For convenience the results have been grouped as follows:

Group I. On twigs of these species the growth of the fungus was as good as or better than the average growth on poplar twigs. Numerous pycnidia were formed all over the stem, within the bark as well as superficially.

Group II. On twigs of these species the growth of the fungus was not equal to that usually obtained on poplar twigs. The pycnidia were fewer and mostly superficial, and they usually developed only at the base of the twigs or on the agar.

Group III. The growth of *V. sordida* on twigs of *Crataegus* sp., and of *V. nivea* on twigs of *Crataegus* sp. and *Tsuga canadensis* Carr. was very poor.

V. sordida	*V. nivea*
GROUP I	GROUP I
Acer rubrum Linn.	*Acer macrophyllum* Pursh.
Acer macrophyllum Pursh.	*Acer saccharum* Marsh.
Acer saccharum Marsh.	*Betula nigra* Linn.
Betula nigra Linn.	*Carpinus Betulus* Linn.
Fagus americana Sweet.	*Toxylon pomiferum* Raf.

Quercus palustris Linn.
Quercus robur Linn.
Quercus Prinus Linn.
Magnolia sp.
Liriodendron Tulipifera Linn.
Toxylon pomiferum Raf.
Rubus sp. (Wild blackberry)
Rosa sp.
Malus glaucescens Rehd.
Malus Niedzwetzkyana Asch. and Graebn.
Pyrus Calleryana Decne.
Ginkgo biloba Linn.
Pinus Strobus Linn.
Pinus sylvestris Linn.
Picea Engelmannii var. *glauca* Beissn.
Juniperus virginiana Linn.

GROUP II

Acer platanoides Linn.
Acer platanoides var. *Schwedleri* Koch.
Betula papyrifera Marsh.
Carpinus Betulus Linn.
Prunus serrulata var. *sachalinensis* Makino.
Pinus nigra Arnold.
Rubus sp. (Wild blackberry)
Rosa sp.
Malus glaucescens Rehd.
Pyrus Calleryana Decne.

GROUP II

Acer rubrum Linn.
Acer platanoides Linn.
Acer platanoides var. *Schwedleri* Koch.
Betula papyrifera Marsh.
Fagus americana Sweet.
Quercus palustris Linn.
Quercus robur Linn.
Quercus Prinus Linn.
Magnolia sp.
Liriodendron Tulipifera Linn.
Prunus serrulata var. *sachalinensis* Makino.
Malus Niedzwetzkyana Asch. and Graebn.
Ginkgo biloba Linn.
Pinus Strobus Linn.
Pinus nigra Arnold.
Pinus sylvestris Linn.
Picea Engelmannii var. *glauca* Beissn.
Juniperus virginiana Linn.

Valsa sordida and *V. nivea* are essentially saprophytes, and they can grow and produce pycnidia on twigs of a wide variety of tree species under cultural conditions, and undoubtedly also under natural conditions. Judging from the excellent growth of *V. sordida* on steamed twigs of many species, and the nature of its facultative parasitism, this species may be expected to cause considerable damage on species other than those already reported. One reason for the lack of information on this point would seem to be the probability that forms of this fungus occurring on other hosts may have been described under different species names.

Lines of Demarcation Between Clons Grown on the Same Plate

Mycelium developed from two or more transfers of the same clon of *V. sordida* or *V. nivea* intermingled when grown side by side. When different clons of *V. sordida* were grown in the same petri dish on poplar decoction agar, a black line of demarcation always formed where the mycelia met (Pl. III, fig. 15). A dense, white ridge of hyphae first developed along the edge of each mycelial mat, leaving a narrow zone between

the two in which relatively few hyphae were present, but finally the two ridges approached and the single ridge then turned black. The distinctness of this line of demarcation varied between different clons. In a few cases there was an imperfect intermingling of the hyphae but the line between the two mycelia was never entirely obliterated.

Clons of *V. nivea* did not form such definite lines of demarcation, and the hyphae along the two adjacent mycelial edges did not pile up. The hyphae along the border were fewer and, as they approached, growth slowed up and finally ceased. The mycelium was somewhat intermingled in the intermediate zone but the fusion was not complete. These inhibitions between clons of both *V. sordida* and *V. nivea* represented various degrees of what Porter (1924) designated as "Type *C* inhibition."

The lines of demarcation cannot be interpreted as evidence of sexual differences in the clons. Miss Cayley (1923) reported that lines of demarcation were formed between some single spore cultures of *Diaporthe perniciosa* Marchal, while other single spore cultures of the same species fused completely. She believes that this mutual aversion is due to the secretion of a toxin by physiologically different strains and that it may have nothing to do with sex. Miss Mounce (1929) obtained similar results in cultures of *Fomes pinicola* (Sw.) Cooke, where lines of demarcation were in some cases even formed between single spore mycelia which united to form clamp connections. Miss Mounce thinks that the influence of physiologically different mycelia upon each other leads to a metabolic upset, and that the density of the lines of demarcation indicates the amount of disturbance. Porter (1924) was able to inhibit the growth of *Helminthosporium* by the use of various chemicals in exactly the same manner as by growing the fungus with other organisms.

The results obtained in this work with clons of *V. sordida* and *V. nivea* seem to bear out the probability of the formation of a toxic substance. The difference in the density of the lines of demarcation between various clons may indicate that the nature or the amount of the toxic substance which is produced is specific for the individual clon (physiological variation).

Inoculation Experiments with Valsa sordida

Technique

During the past 4 years many series of inoculations have been made with pycnospores and mycelium of *V. sordida*. The inoculum was in all cases of single pycnospore origin from material collected in Maine, New York, or Quebec. Ascospore cultures were not available for these experiments. Two general methods were used in making pycnospore inoculations. Where it was desired to put spores on a very limited area of the plant, a spore suspension in sterile water was applied with a small wire loop. When larger areas were to be inoculated the spore suspension was sprayed on with an atomizer.

The following method of mycelial inoculation was found very satisfactory and gave very uniform results. Gelatine capsules no. 000 were sterilized by immersion in 95 percent alcohol for 15–20 minutes and then dried under a sterile paper. A few drops of sterile poplar decoction liquid were put into a capsule, a bit of mycelium was added, and the capsule was quickly closed, dipped into melted paraffin, and laid aside to drain. When the first coating of paraffin had become hard, a second and then a third were added. After 2 or 3 days the mycelium had usually grown over the inner surface of the capsules and they were then ready for use. The inoculated capsule was cut longitudinally with a sterile razor blade and one half was fastened over the spot where the inoculation was to be made, usually a knife wound in the bark. It was first shaped to the stem and tied in place with string, and then sealed on by pouring melted paraffin around the edges. A small amount of lanolin added to the paraffin made it easier to work with. The other half of the capsule was put on agar to test the viability of the inoculum contained in it.

Inoculations on Plants in the Greenhouse

Plants for greenhouse experiment were grown from 10-inch cuttings which were started in sand and transplanted to rich soil in individual pots when they were rooted. All pots were watered from the bottom. Over a period of three years a total of approximately 2000 plants were used in the greenhouse experiments. The following kinds of poplars were used: *P. balsamifera virginiana* Sarg. (1919), *P. tacamahacca* Mill. (as interpreted by Sargent, 1919), *P. tremuloides* Michx., *P. grandidentata* Michx., and the hybrid clons Eugenei, Norway, and Regenerata. *P. tremuloides* and *P. grandidentata* root with great difficulty, and when they were used in these experiments they were in such poor growing condition that the mortality was invariably 100 percent very soon after inoculation. *P. balsamifera virginiana* and *P. tacamahacca* also rooted poorly and the mortality after inoculation was very high. The Eugenei, Norway and Regenerata poplars rooted readily, and for this reason the following results were based upon work with these clons.

Pycnospore Inoculations

Plants in Inoculation Chambers. One series of plants was kept in the "iceless refrigerator" type of inoculation chamber described and pictured by Hunt (1919). Cuttings planted in November or early December did not begin to grow until January, and if they were inoculated when planted, from 80–100 percent were killed before active growth of the cuttings began. The infection usually started at the upper cut end, and the progress of the disease could often be followed down the stem. Cuttings planted after the middle of January started to grow within one or two weeks. When such cuttings were inoculated at the time of planting, the mortality varied from 40–75 percent but practically all of the cuttings became infected at

the upper end above the last bud (Pl. IV, figs. 4, 5). When well rooted cuttings in soil were inoculated, the mortality was less than 5 percent, but over 75 percent of the plants became infected at the upper end. In all cases where plants became pot-bound, or growth was poor for any other reason, the mortality was much higher than among plants growing under better conditions. Occasionally the tip of a shoot or a leaf of an apparently vigorous plant which had been sprayed with spores became blackened, the dead area progressing until the entire shoot was killed. Tissue cultures from the inside of such shoots usually gave *V. sordida*, but in some cases the fungus was lacking.

Where the inoculations were made on wounds, small cankers soon developed around these wounds in over 80 percent of the cases. In order to determine whether this fungus is essentially a wound parasite, cuttings which showed no bark injury were selected, and the upper ends were covered with paraffin before inoculation. Only 3 out of 50 such cuttings became infected, and it seemed probable that in these three cases the infections entered through minute wounds which had been overlooked.

Plants on the Greenhouse Bench. The percentage of infection and mortality resulting from pycnospore inoculations was much more erratic, and usually considerably lower, when the inoculated plants were kept on the greenhouse bench. This was to be expected because of the much lower humidity under which the plants on the bench were growing. Tips of young shoots or young leaves were never killed on inoculated plants outside the humidity chambers.

Mycelial Inoculations

All plants on which mycelial inoculations had been made were kept on the greenhouse bench. The mycelial inoculations on wounds in the bark gave very uniform results. Inoculations in November and early December resulted in 90–100 percent mortality. When such inoculations were made later, after the middle of January, the percentage of infection depended upon the point of inoculation. All cuttings inoculated above the top bud became infected, but when the inoculation was lower the effect on the individual cutting differed. Cuttings of small diameter were most often killed, at least above the point of infection. Vigorous, large diameter cuttings either showed no outward sign of injury or developed only small cankers. Inoculations were also made on uninjured bark but only a very few of such inoculations caused infection.

Field Inoculations

Pycnospore Inoculations

Field inoculations were made on young trees of *P. grandidentata* and *P. tremuloides* from one-fourth of an inch to 3 inches in diameter. Pycnospore inoculations on uninjured bark failed to cause infection. Spores

sprayed on new wounds resulted in from 20–50 percent infection with a high percentage of mortality among the weaker trees. The appearance and the development of the infections resulting from spore inoculations were the same as in the case of the mycelial inoculations described below. The writer has been unable so far to infect living leaves, either wounded or unwounded, by spore inoculations in the field.

Mycelial Inoculations

Mycelial inoculations were made over fresh wounds on stems of one- and two-year-old trees of *P. tremuloides* and *P. grandidentata* growing wild in Oxford County, Maine. Inoculations were made during the first week of March, 1927, and 10–15 trees were inoculated every week until the end of May. During the summer of 1927 about 50 percent of the trees under three-fourths of an inch in diameter, and about 5 percent of the larger, more vigorous trees were killed by the fungus. There were cankers of various sizes around the point of inoculation on some of the slower growing trees, but on most of the vigorous trees the wounds appeared to be healing normally and there was no outward sign of infection. During 1928 more of the cankered trees died, and in all cases pycnidia of *V. sordida* developed on the dead stems. The wounds on the most vigorous trees had healed entirely, but on the less vigorous individuals larger or smaller open scars had formed around the point of inoculation. A very few trees with well-defined cankers which were still living in the fall of 1928 were killed by the fungus during 1929. By the fall of 1929 the inoculation wounds and small infection scars had healed on most of the trees which were still alive, but the slower growing trees still had open scars which often looked much like small insect burls (Pl. V, fig. 1).

During March, 1930, the inoculated trees which were still living were cut and sectioned longitudinally through the point of inoculation. There was a region of brown discoloration in the wood and pith of all these trees, which extended up and down the stem from the point of inoculation and also out into the side branches which were within the area. The brown color was usually limited to the wood ring of the year of inoculation and to one or two rings each side of it. The discoloration in the wood extended longitudinally as much as 12–14 inches, and in the pith often as much as 20–24 inches, to each side of the inoculation point. The infection in the wood was mostly on the inoculated side of the stem, but where the bark and wood had overgrown the label wire, the infection in the wood followed the wire entirely around the stem (Pl. V). Tissue cultures were made from the infected wood and pith of a number of these trees, and over 90 percent of such cultures produced *V. sordida*.

The inoculation experiments indicate that *V. sordida* is a facultative wound parasite, and that the injury to the plant is most severe during the dormant period of the tree. Infection through young living shoots and

leaves can hardly be of great importance under natural conditions, since only occasional young shoots on plants in the humidity chambers were killed. Such moisture conditions would not exist under natural environmental conditions, and the fact that cultures from some of the dead shoots did not produce *V. sordida* may indicate some other primary, or at least contributing, cause of death. Inoculations on living twigs or leaves in the field have been unsuccessful. The fact that the greatest percentage of infection from pycnospore inoculation was obtained on plants in the humidity chambers, may indicate that in the field the greatest amount of spore infection takes place during long periods of rainy weather.

Experimental Evidence on Correlation between Vigor of Tree and Extent of Fungus Injury

In the fall of 1926 fifteen one-year-old rooted trees of *P. balsamifera virginiana* and of the Eugenei poplar, bearing pycnidia of *Valsa sordida* at the upper dead end of the original cuttings, were dug from a poor site on which they were growing and planted in rich garden soil. During 1927 and 1928 all the transplanted trees grew very vigorously and apparently recovered from the disease. Although the dead stubs of the original cuttings were still present after two years, there were no cankers on any of these trees (Pl. IV, figs. 1, 2). The diseased control trees which had not been transplanted from the unfavorable site were all killed by the fungus, whereas healthy trees growing on the same site were still alive but had grown only 2 or 3 feet in height.

In the fall of 1928 the stems of some of the trees which had recovered were split longitudinally through the old cutting. The wood of the original cutting was a dark, red-brown color. This discoloration extended slightly into the adjacent wood, and in the pith it extended for some distance into the living stem and side branches (Pl. IV, fig. 3). *Valsa sordida* was cultured from the discolored wood and pith of all the apparently healthy trees.

Discussion and Summary

The genus *Valsa* includes about 135 described species of chiefly saprophytic fungi. A few of the species, however, are known to be facultative parasites, and of these *Valsa sordida* and *V. nivea* are to be recognized as causing much injury to poplars. The identity and relationship of the spore stages of these two fungi are now certain. The mycelia of single ascospore cultures of *Valsa sordida* have always produced the pycnidial stage known as *Cytospora chrysosperma*. Likewise the pycnidial stage known as *Cytospora nivea* was always obtained from single ascospore cultures of *Valsa nivea*. These two species live readily on dead wood, but they also produce cankers on living trees. *Valsa sordida*, which is apparently the more vigorous parasite, has been found on all kinds of poplar in New England and New York. It is commonly parasitic on the native aspens, *P. tremuloides* and *P. grandidentata*, and causes serious damage to the Lombardy poplar.

The fact that *V. sordida* and *V. nivea* have been grown on various agar media and on twigs of 29 species of plants, indicates that they may live on rather widely divergent substrata as saprophytes. As is quite commonly the case, both the ascospores and the pycnospores of *V. nivea* swell excessively and become spherical before they germinate. In culture, the range of temperatures required for growth is more limited for *V. nivea*, its mycelium always grows more slowly than that of *V. sordida* under comparable conditions, and it is less dense and darker in color. The ascospores and pycnospores of *V. sordida* germinate more rapidly than those of *V. nivea*, but they do not become spherical, and the mycelium is usually white, becoming dark colored only in the older portions of the cultures.

In *Valsa sordida* especially, different types of growth and general appearance were observed among cultures from single ascospores obtained from the same canker. There were apparently inherent differences in the rate of growth, the amount of aërial and surface mycelium, and the earliness of pycnidial formation, but these differences were most marked on poplar decoction agar, upon which growth was fairly rapid. Many matings of different clons were made, and where the mycelia met, lines of demarcation were always formed by retardation of growth and a piling up of hyphae. This is the so-called "antagonism" frequently observed by other investigators. No perithecia were formed in these or in any other cultures and hence no proof was obtained for heterothallism in this species. It is evident, however, that single ascospore cultures give different types of mycelia, a fact which suggests that there are various strains of *Valsa sordida* in existence. There is a possibility that these may differ in specificity as to host and in pathogenicity.

Inoculations were made with spores and mycelium of *V. sordida*, and these readily produced infection of young branches and of trunks of young trees, but only through wounds in the bark. Following inoculation two extreme types of results were obtained, apparently depending on the vigor of the host. In most cases on trees of poor growth, the fungus grew in the bark and wood, killing the cambium, and often the branch or even the entire young tree was killed. On vigorously growing trees either the wound healed, or a small canker was formed which subsequently healed over, but in either case the wood within was brown in color, and tissue cultures taken from this wood as late as two years after inoculation gave living mycelium. Further, the virulence of the disease was controlled experimentally by transplanting infected trees which were growing poorly, due to poor soil conditions, to a site which was favorable to vigorous growth. The control trees which were not transplanted were all killed, but the transplanted trees grew vigorously and apparently recovered from the disease. The mycelium of the fungus was, however, still alive in the wood of these trees two years later.

Although poplars of all ages are subject to infection by *V. sordida*, it often happens that some trees are badly diseased or even killed by the fungus while other individuals close by are perfectly healthy. This seems to be due (1) to the accidents of wound infection, especially the occurrence of injuries which produce dead wood on the tree, such as insect attacks, ice breakage, etc., and (2) to differences in vigor of growth of the host. It is also possible that certain poplar clons or seedling individuals may be more resistant than others to attack by *V. sordida*.

The writer wishes to express his gratitude to Dr. A. B. Stout under whose direction these investigations were carried out. The work was done in connection with a project of forestation in which the Oxford Paper Company and the New York Botanical Garden are coöperating. The writer is also indebted to Dr. R. A. Harper and to Dr. B. O. Dodge for valuable help during the progress of this work, and to Dr. J. H. Barnhart for special assistance in evaluating the taxonomic literature.

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EXPLANATION OF PLATES

PLATE I

FIGS. 1–5. *Valsa nivea*. FIG. 1. Three perithecial fruiting bodies on bark of *P. tremuloides*. Ostioles of perithecia can be distinguished. × 14. FIG. 2. Section through a perithecial stroma showing the stromatic "conceptacle." × 12. FIG. 3. Mature perithecia. × 14. FIG. 4. Four pycnidial fruiting bodies on bark of *P. tremuloides*. × 15. FIG. 5. Section through a pycnidial stroma showing the stromatic "conceptacle" and the central pore. × 12.

FIGS. 6–11. *Valsa sordida*. FIG. 6. Perithecial fruiting body with ostioles of perithecia grouped around a central disk. × 10. FIG. 7. Ostioles of perithecia grouped irregularly, central disk lacking. × 10. FIG. 8. View of underside of perithecia, bark

tissue removed. Perithecia dry, collapsed, still attached to epidermis at the ostioles. × 12. FIG. 9. Mature perithecia. × 14. FIG. 10. Surface view of three pycnidial fruiting bodies on bark of *P. tremuloides*. × 14. FIG. 11. Section through a pycnidial stroma. × 14.

FIGS. 12–19. Germination of ascospores and pycnospores, sown on poplar decoction agar in the same petri dish and kept at room temperature. × 350. FIG. 12. Ascospores of *V. nivea* 24 hours after sowing. FIG. 13. Ascospores of *V. nivea* 46 hours after sowing. FIG. 14. Ascospores of *V. sordida* 24 hours after sowing. FIG. 15. Ascospores of *V. sordida* 46 hours after sowing. FIG. 16. Pycnospores of *V. nivea* 24 hours after sowing. FIG. 17. Pycnospores of *V. nivea* 46 hours after sowing. FIG. 18. Pycnospores of *V. sordida* 24 hours after sowing. FIG. 19. Pycnospores of *V. sordida* 46 hours after sowing.

PLATE II

FIG. 1. Lombardy poplar killed by *V. sordida*. Tree five years old, seventeen feet high, three inches in diameter at base, growing on a poor site. Numerous branches killed during the three years previous to death of the tree.

FIG. 2. Trunk of above tree (fig. 1) showing old cankers and dead sucker shoots.

FIG. 3. Pycnidial sporehorns of *V. sordida* developed on dead twigs from above tree (fig. 1). Twigs kept in moist chambers.

FIGS. 4 and 5. Stems of two-year-old trees of *P. tacamahacca*, about 1.5 inches in diameter, with cankers of *V. sordida*.

FIG. 6. Cross-section of stem at middle of canker (fig. 5), showing discolored infected wood.

FIG. 7. Cross-section of stem ten inches below lower end of canker (fig. 5) showing extent of infection in wood.

FIG. 8. Old canker on a living limb of *P. nigra*. Limb three and one-half inches in diameter. Canker extended along upper side of limb almost to main trunk.

FIG. 9. Longitudinal section of limb (fig. 8) showing discolored, infected wood and pith extending into apparently healthy branches on the infected limb.

FIG. 10. Cross-section of limb (fig. 8) showing extent of infected wood.

PLATE III

FIGS. 1–12. Mycelium of three different clons (single ascospore cultures) of *V. sordida* on four kinds of agar media. Photographs taken twelve days after inoculation. Cultures kept at 25° C. FIG. 1. Clon S-145 on poplar decoction agar. FIG. 2. Clon S-145 on corn meal agar. FIG. 3. Clon S-145 on potato dextrose agar. FIG. 4. Clon S-145 on synthetic nutrient agar. FIG. 5. Clon S-155 on poplar decoction agar. FIG. 6. Clon S-155 on corn meal agar. FIG. 7. Clon S-155 on potato dextrose agar. FIG. 8. Clon S-155 on synthetic nutrient agar. FIG. 9. Clon S-176 on poplar decoction agar. FIG. 10. Clon S-176 on corn meal agar. FIG. 11. Clon S-176 on potato dextrose agar. FIG. 12. Clon S-176 on synthetic nutrient agar.

FIG. 13. Two types of mycelial growth observed among clons of *V. nivea*. S-98 at bottom and S-102 at top of dish. Both from ascospores from the same source. Culture on poplar decoction agar.

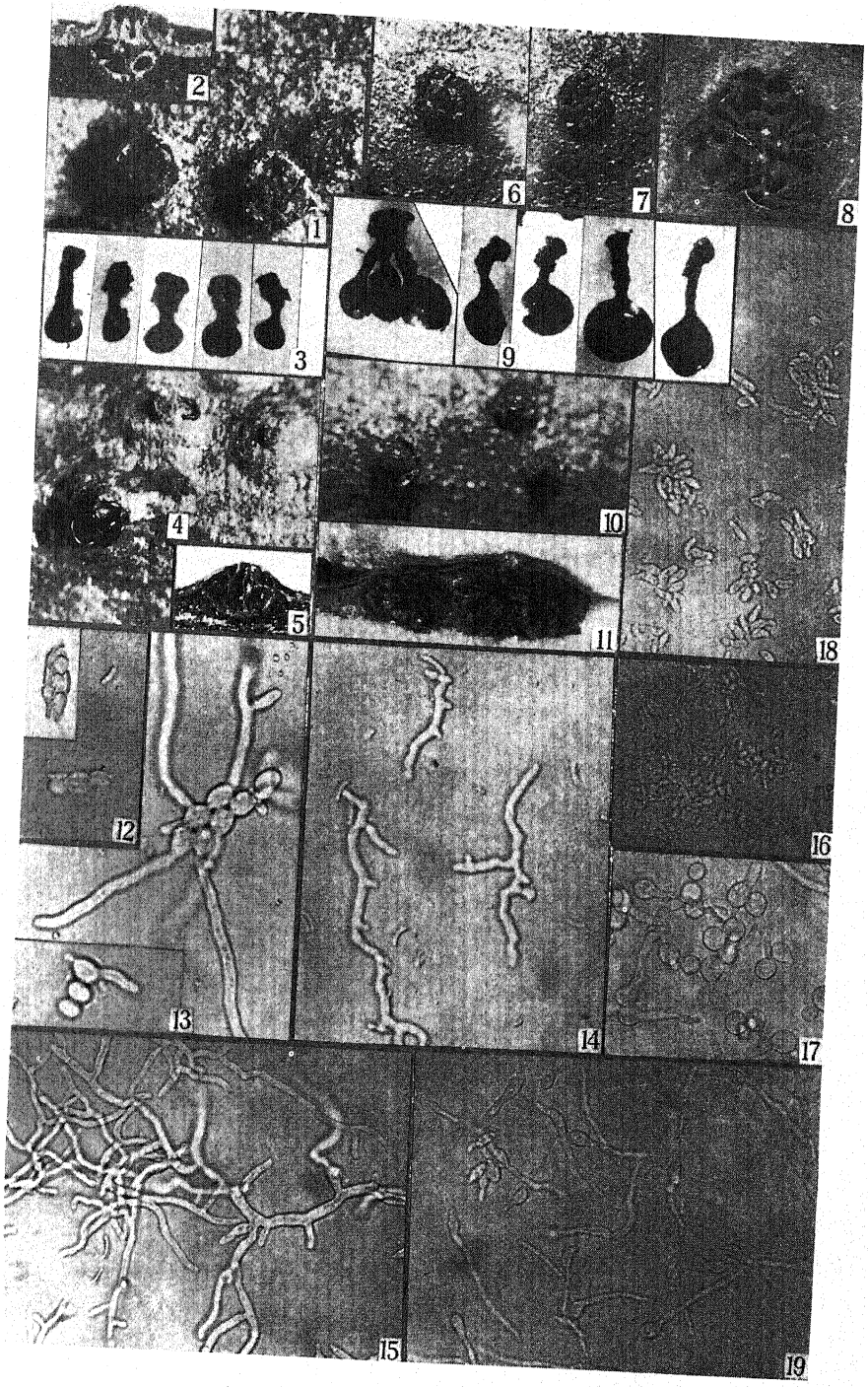
FIG. 14. Two clons of *V. sordida*, S-145 (bottom) and S-155 (top) growing on poplar decoction agar in same plate, line of demarcation just beginning to form.

FIG. 15. Lines of demarcation between four different clons of *V. sordida* growing on poplar decoction agar.

PLATE IV

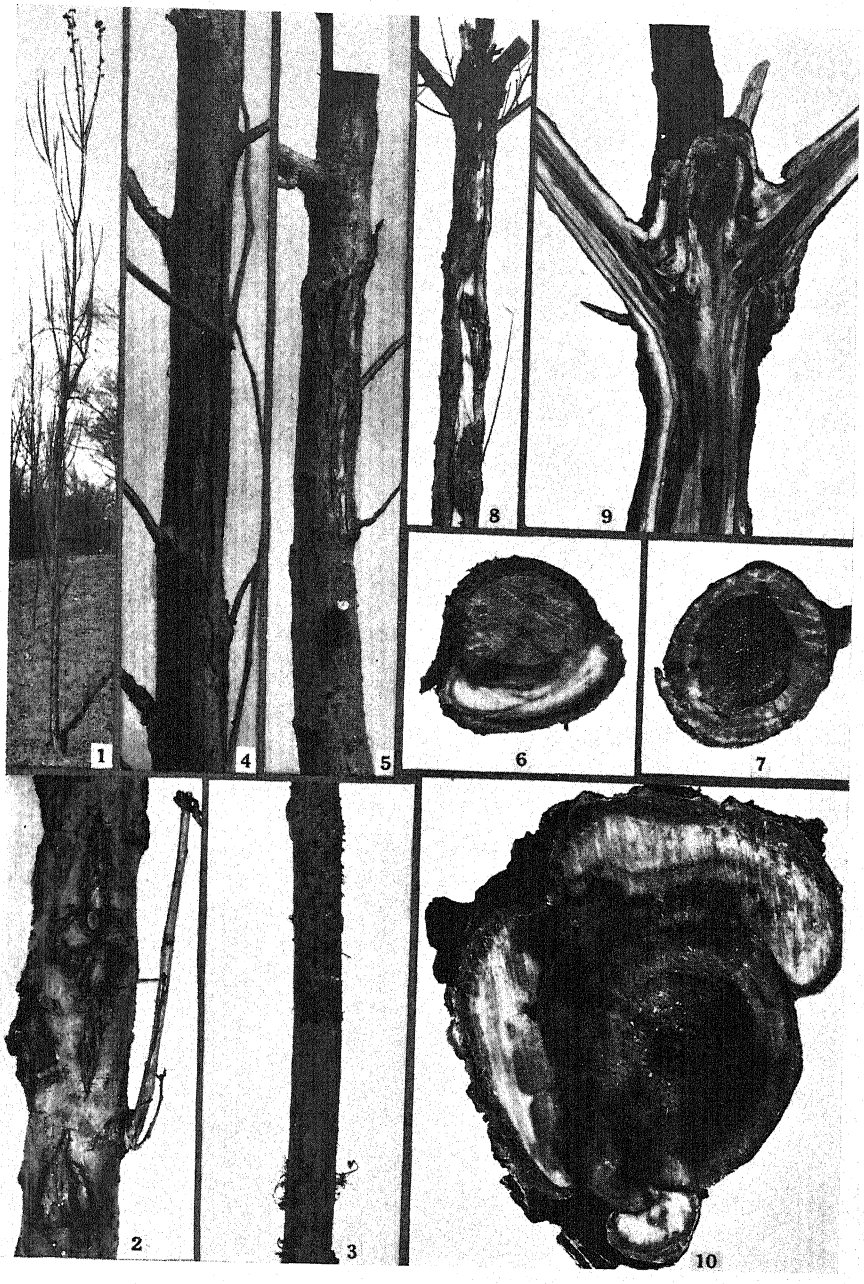
FIG. 1. One-year-old rooted tree growing on a poor site, infected by *V. sordida* at top of original cutting.

FIG. 2. One of the same lot of infected trees as that in figure 1, two years after transplanting to a better site. Tree twelve feet high and apparently recovered from the disease.



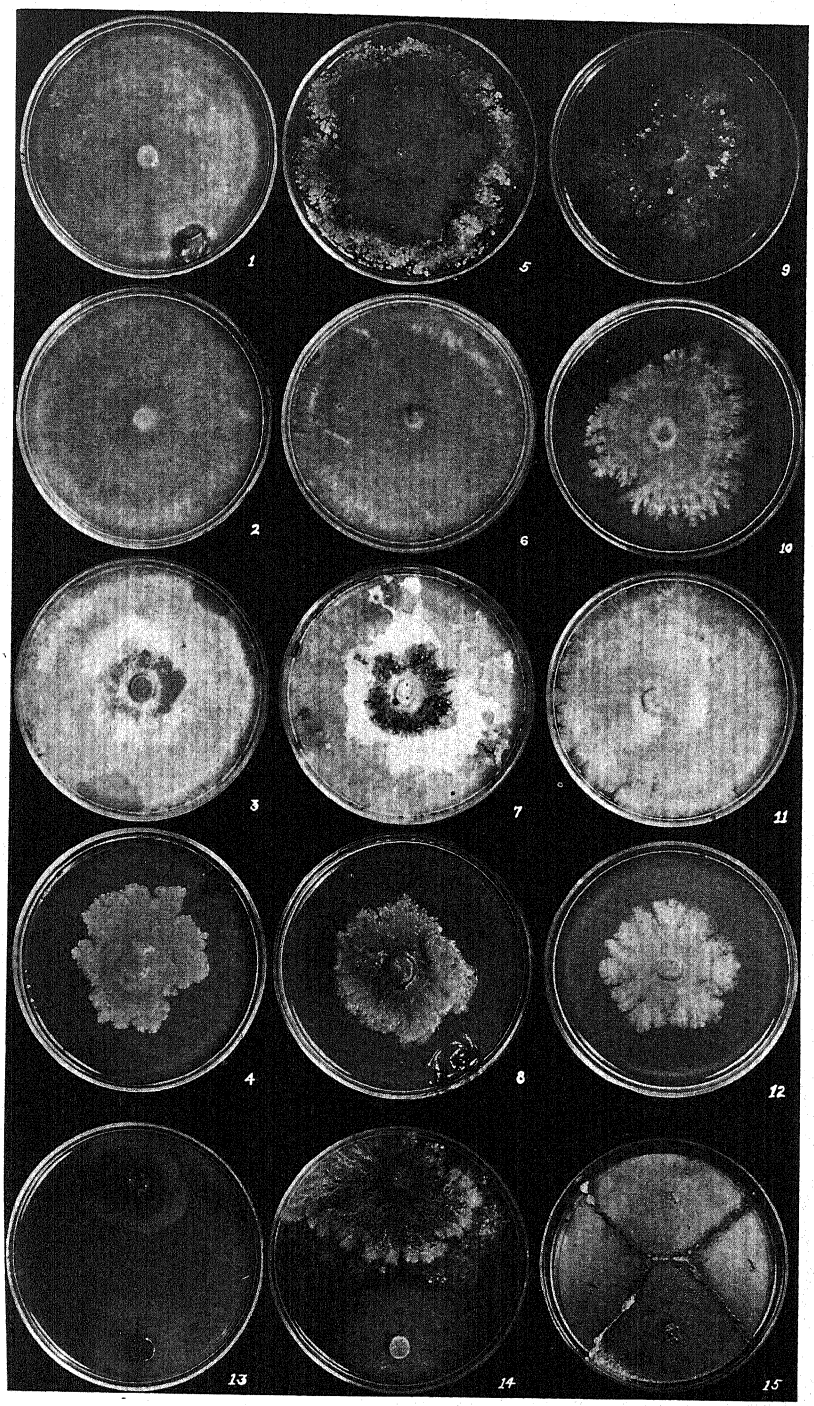
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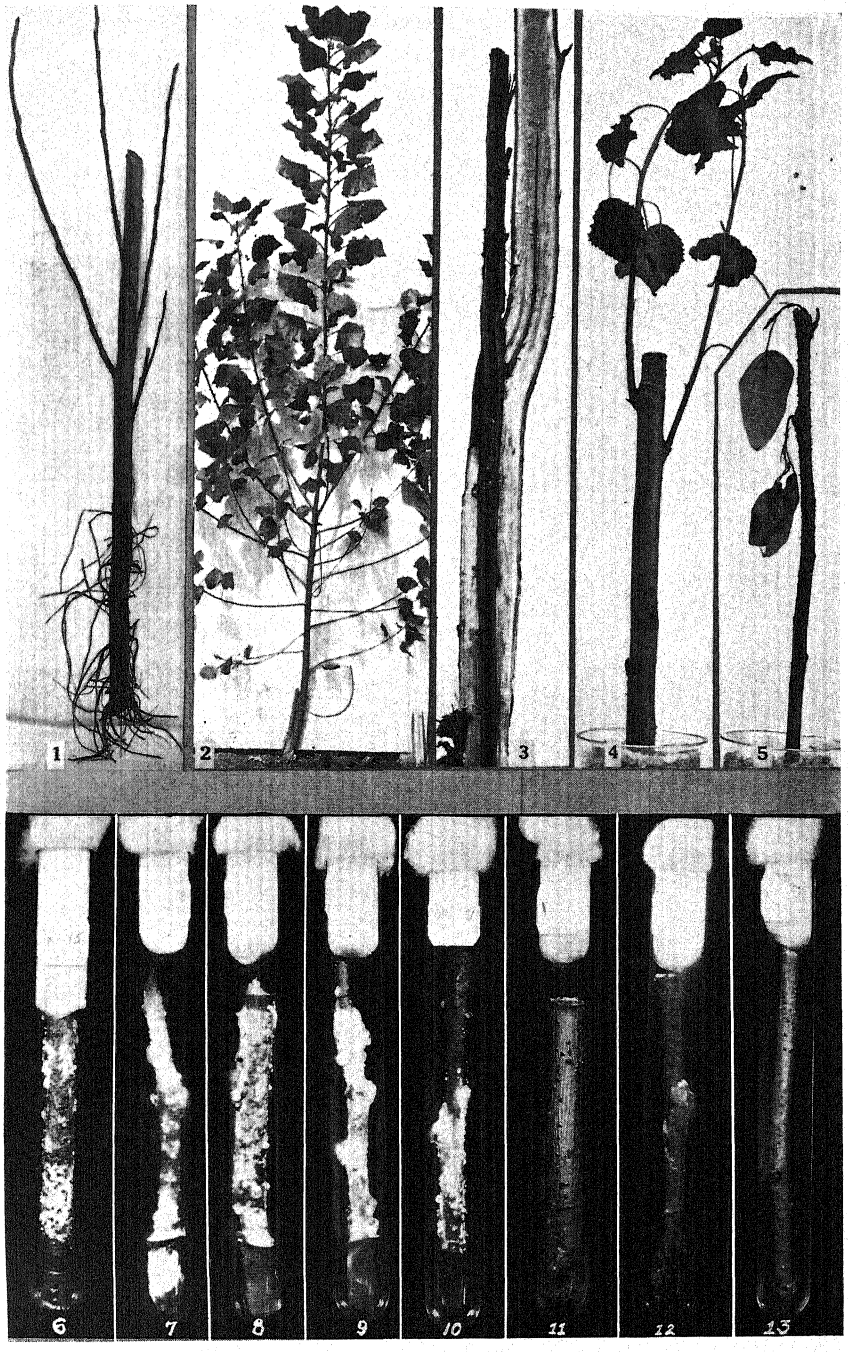


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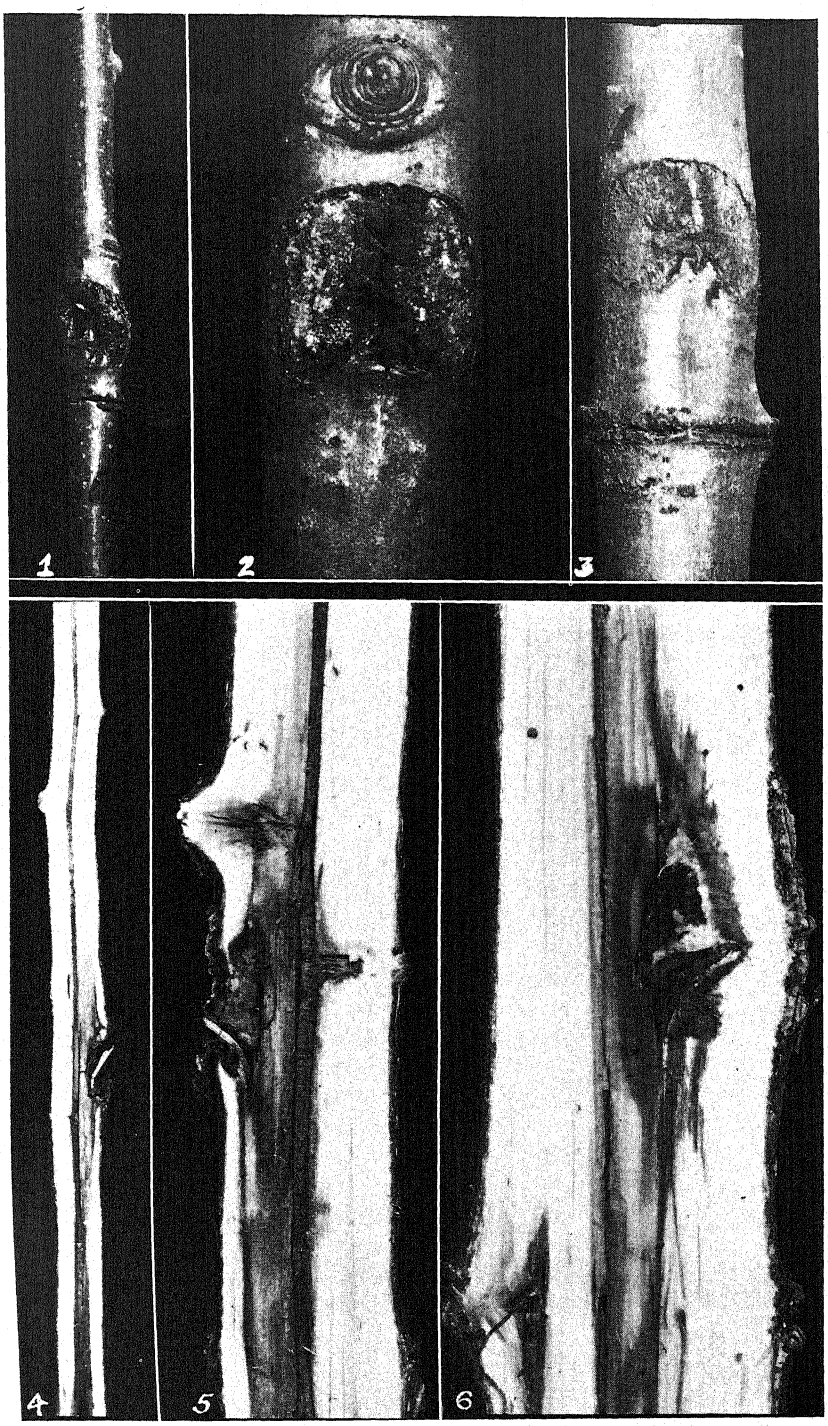


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FIG. 3. Section through butt of tree shown in figure 2. Discolored, infected wood and pith extends into main trunk and into root. *V. sordida* obtained in cultures from the discolored wood and pith.

FIGS. 4 and 5. Two cuttings planted in the greenhouse early in January and inoculated immediately. Norway poplar (fig. 4) rooted readily, infected only at upper end. *P. tacamahacca* rooted with difficulty, killed by fungus.

FIG. 6. Growth of *V. sordida* on steamed twigs of Norway poplar.

FIG. 7. Growth of *V. sordida* on steamed twigs of *Picea Engelmannii* var. glauca.

FIG. 8. Growth of *V. sordida* on *Pinus Strobus*.

FIG. 9. Growth of *V. sordida* on *Pyrus Calleryana*.

FIG. 10. Growth of *V. sordida* on *Quercus palustris*.

FIG. 11. Growth of *V. nivea* on *Norway poplar*,

FIG. 12. Growth of *V. nivea* on *Quercus Robur*.

FIG. 13. Growth of *V. nivea* on *Gingko biloba*.

PLATE V

Three stems of *P. tremuloides* inoculated with mycelium of *V. sordida* in 1927, photographed and sectioned March 1930.

FIG. 1. Stem three-quarters inch in diameter, with a small canker at the point of inoculation.

FIG. 2. Stem one and one-half inches in diameter, with a partially healed inoculation wound.

FIG. 3. Stem one and one-half inches in diameter, with a completely healed inoculation wound. (Note: The "girdle" below the inoculation point in No. 1 and No. 3 was caused by the label wire).

FIG. 4. Section through stem in fig. 1. The infection extended for a considerable distance in the wood and pith.

FIG. 5. Section through stem in fig. 2. Infection in wood and pith extended into the branch just above point of inoculation.

FIG. 6. Section through stem in fig. 3. The infection in this stem was entirely sealed in. Fungus followed label wire around the entire stem. *Valsa sordida* was cultured from the discolored wood and pith of these stems.

A MICROCHEMICAL STUDY OF SOYBEANS DURING GERMINATION [1]

FLOYD W. VON OHLEN

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INTRODUCTION

A review of the literature on the germination of seeds shows a growing need of further studies of the chemical changes that occur during germination. Both macrochemical and microchemical methods of study have been used, but the majority of the work has been macrochemical. Choate (4) and Toole (22) have reported microchemical work on the germination of starchy seeds. The present investigation has been an attempt to use microchemical tests as a means of following the chemical changes that occur from the time an oily seed begins to germinate until the seedling dies because of the depletion of reserve foods.

HISTORICAL

Miller (9) gives a very thorough review of the early literature on the germination of oily seeds. In his own work with sunflower seedlings he found that the depletion of the oily reserves in the cotyledons began at the end next to the hypocotyl and gradually advanced toward the opposite end, the most marked change occurring between the fifth and tenth days. He was of the opinion that the oily reserve broke down, in part at least, to glycerine and free fatty acid. Only a small amount of sugar was found in the cotyledons, although it was abundant in the hypocotyl. He states that the sugar produced from the oil was the material used in the plant in the formation of new cell walls in the growing parts.

Choate, working with germinating wheat, reported dextrin in the scutellum and coleorhiza, and starch in the root cap after ten hours in the germinator. Reducing sugar appeared in the embryo by the eighteenth hour. The protein content of the endosperm, except that in the aleurone layer, decreased rapidly during germination. Asparagine appeared in the coleoptile on the fourth day, and a little later in the root.

In germinating corn Toole did not find reducing sugar in the embryo until the seed had been in the germinator for twenty hours and then only in the tip of the coleorhiza. Later it was found in all growing parts except the embryonic tissue. Reducing sugar appeared in the endosperm a little later than in the embryo. Only a small part of the endosperm reserves had been used at the end of seven days. The depletion began next to the

[1] Papers from the Department of Botany, The Ohio State University, No. 261.

scutellum and progressed into the endosperm. He reported that there was evidence of the movement of soluble protein substances from the endosperm toward the embryo. The intensity of the biuret reaction increased in the active regions of the embryo at the beginning of germination. This reaction gave a pinkish-purple color in the vascular tissue of the scutellum, while the other tissues of the scutellum gave a dull blue color reaction.

Yocum (24) who worked with the translocation of food materials in wheat seedlings found that the ether extract disappeared from the seeds more slowly than do the carbohydrates and nitrogen during the early stages of germination. Sugar increased rapidly in the seeds the first six days, and then decreased. The translocation of sugar to the plumules and roots was more rapid than its use, as long as there was a supply of starch in the seeds. During the first six days nearly three-fourths of the starch of the seeds had been used, and after the ninth day only one percent remained. Nitrogen was translocated rapidly in the three-day-old seedlings. The ash content of the plumules and radicles reached its maximum between the twelfth and fifteenth day.

Jodidi (5), working with etiolated corn seedlings, found that up to 48 percent of the proteins of the corn seed was converted into water-soluble nitrogen compounds within the first eight days of germination. During the same period there was a steady increase of acid amide and amino nitrogen, while humin and polypeptide nitrogen decreased. He thought that the acid-amides increased at the expense of the amino acids, which in turn were formed from the polypeptides. He considered that polypeptides, along with proteoses, were among the first products of the degradation of proteins.

Sachs (15) made microchemical studies of a number of oily seeds during germination. He concluded that part of the oily reserve in the seed was transformed into starch, that starch was not a transition product between oil and sugar, and that oil was not first changed to sugar and then to starch.

A summary of the literature on the macrochemical analyses of oily seeds and resulting seedlings reveals that most workers are of the opinion that the oil in oily seeds breaks down into glycerine and fatty acid during germination, and that carbohydrates are finally formed. Starch appears or increases in a large number of oily seeds during germination. Reducing sugar appears in the embryo and is usually quite abundant in the developing seedlings. Proteins change rapidly to a soluble form and asparagine appears in the seedling by the third or fourth day.

Methods

Manchu soybeans obtained from Dr. H. L. Borst of the Farm Crops Department of the Ohio State University were used as experimental material. For the study of the first stages of germination the seeds were soaked for one hour in distilled water and were then germinated between

wet paper towels. The temperature ranged from 21° to 25° C., although most of the time it was 22° or 23° C.

The older seedlings studied were grown in two-inch pots filled with moist quartz sand. The seeds were soaked before planting and the pots kept in the dark except during the process of watering the seedlings.

All sections were cut free hand with a razor, except those of the cotyledons which were all cut to the thickness of 75 micra with a freezing microtome. For the first five days all parts of the seedling were sectioned and tested, but in older seedlings sections were cut only from the base of the root, the apex of the hypocotyl, the base and apex of the epicotyl, and the cotyledons.

The microchemical tests used are those found in Molisch (10) and in Eckerson's "Outlines of Microchemistry," with the exception of the tests for organically bound phosphorus and magnesium which were those developed by Klein (6, 7). All the results given are the average of a large number of tests.

In all of this work the phosphorus and magnesium that did not react with the reagents without the tissue having been previously treated with an oxidizing agent, as described by Klein, were considered as organically bound. The term "oil" in this paper applies only to that portion of the oily substance contained in the seed or seedling which will dissolve in acetone.

The sodium cobalt nitrite test was used for determining the presence of potassium. Platinum chlorid failed to give a positive test on fresh sections, but a copious precipitate of potassium chloroplatinate crystals was obtained on ashed sections. When sections of cotyledons were washed in water for two days, so much of the potassium leached out that none of the reagents would give a positive test for it even after the sections were ashed. This showed that the potassium compounds in the sections were water soluble. The cause of the lack of the formation of potassium chloroplatinate crystals in fresh sections of the cotyledons was not determined. Subsequent treatment of the sections with alcohol also failed to induce any crystal formation except that of the reagent, though the writer had previously found this procedure to work well on sections of tomato stem.

Microchemical Study

Ungerminated Seed

Cotyledons. A small amount of starch was found in the palisade tissue of the cotyledons. No reducing sugar was detected, although there was considerable non-reducing sugar. The Flückiger test used to detect reducing sugar was found to be sensitive to .0009 percent of glucose in water. Its sensitivity when applied to a plant section was not determined. All cells contained a large amount of protein and oil reserves. Most of the

phosphorus and magnesium was organically bound. Only traces of inorganically bound phosphorus and magnesium were found, while potassium was quite abundant.

Plumule. Starch and reducing sugar were not found in the plumule. Some non-reducing sugar, considerable oil, and a large amount of protein were present. Strong tests for potassium, inorganically bound phosphorus, and magnesium were obtained, but only fair tests for organically bound phosphorus and magnesium.

Hypocotyl. A small amount of starch was scattered throughout the hypocotyl, with the exception of the end from which the root developed. No reducing sugar was detected, but some non-reducing sugar and considerable oil was found. The greatest concentration of the last two substances was in the apex of the hypocotyl. Strong reactions for proteins were obtained with the biuret and Millon tests. A large amount of potassium and fair amounts of both forms of phosphorus and magnesium were present.

Thus, a small amount of starch was found in the cotyledons and hypocotyl. Reducing sugar was not detected in any part of the seed. Non-reducing sugar was present in all parts of the seed, being more abundant in the cotyledons and the apex of the hypocotyl than in the plumule and base of the hypocotyl. Both protein and oil were abundant in all parts of the seed. No asparagine was detected in any part of the seed. Both organically bound phosphorus and magnesium were found in all parts of the seed, being quite abundant in the cotyledons and the apex of the hypocotyl. Both inorganically bound phosphorus and magnesium were quite abundant in the plumule; smaller amounts were found in the hypocotyl, and only traces in the cotyledons. Potassium was abundant in all parts of the seed.

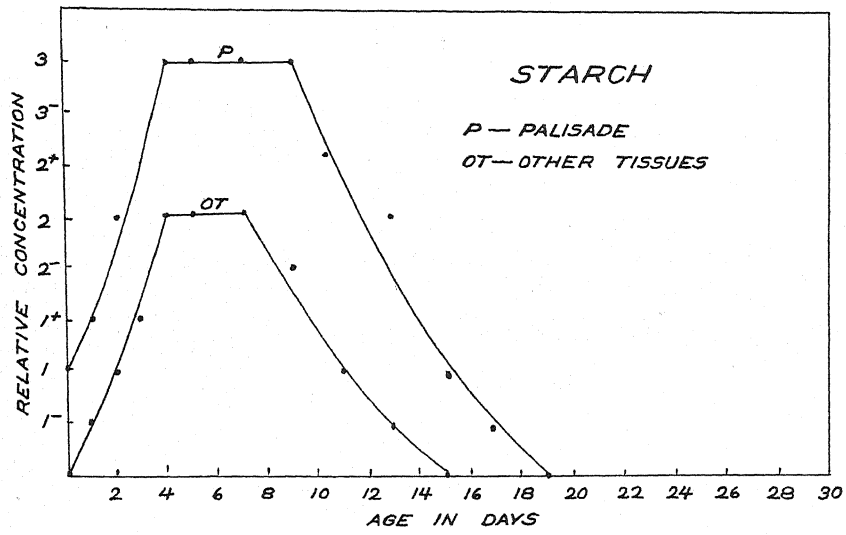
Transformation and Translocation During Germination and Growth of Seedlings

Cotyledons

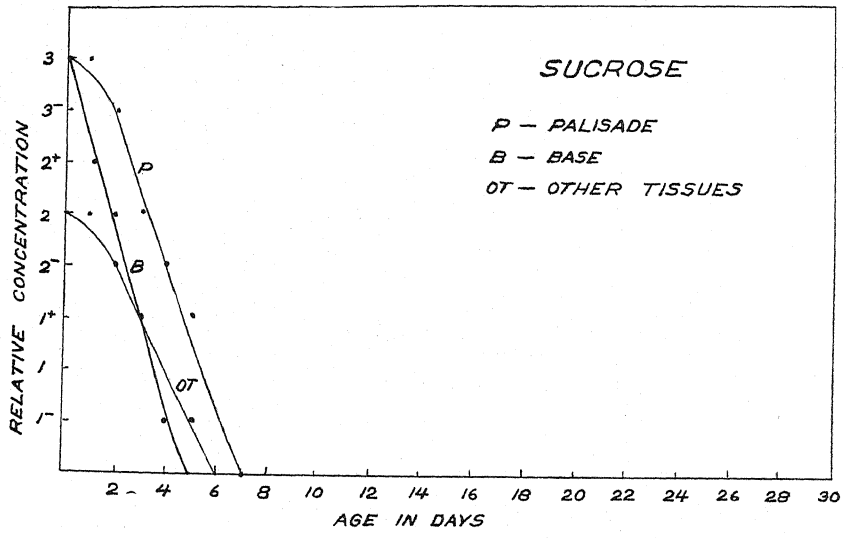
Carbohydrates. There was a gradual increase in the amount of starch in the cotyledons from the first to the fifth day. On the ninth day there was an evident decrease of starch in all parts of the cotyledons except the palisade. It was detected only in the palisade and in the starch sheaths of the larger veins by the seventeenth day and this disappeared during the next two days (see series *C* of Pl. VI.)

A small decrease in the amount of non-reducing sugar had taken place by the end of the second day, and by the seventh day the cotyledons had been depleted of all of this sugar (see series *B* of Pl. VI). A slight reduction of the Flückiger reagent was obtained on the fourth and fifth days in the vascular system at the base of the cotyledons. This was the only indication of reducing sugar obtained in the cotyledons during the entire study.

Proteins and Oil. The intensity of the biuret reaction changed from a dark to a light purple by the fifth day, and to a very light purplish blue by the twenty-ninth day. The Millon reaction changed from dark red on



TEXT FIG. 1. A graphic representation of the changes of starch content in the cotyledons.

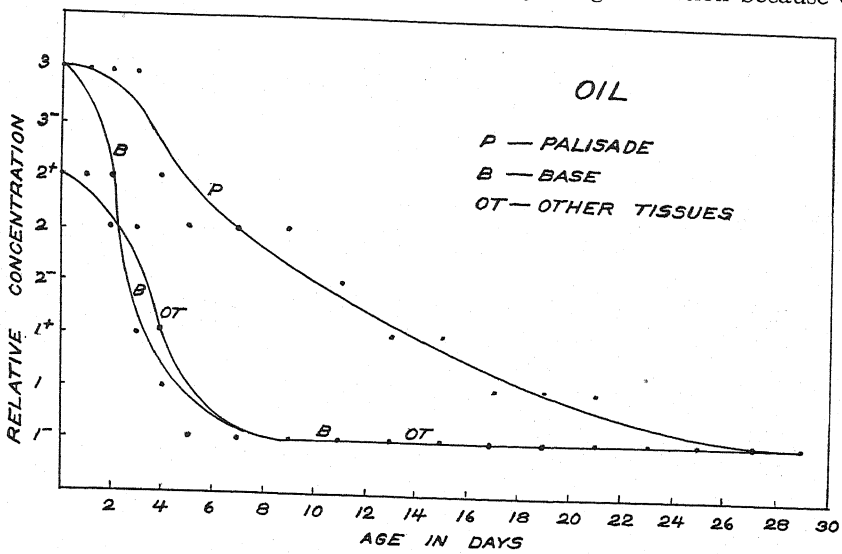


TEXT FIG. 2. A graphic representation of the changes of sucrose content in the cotyledons.

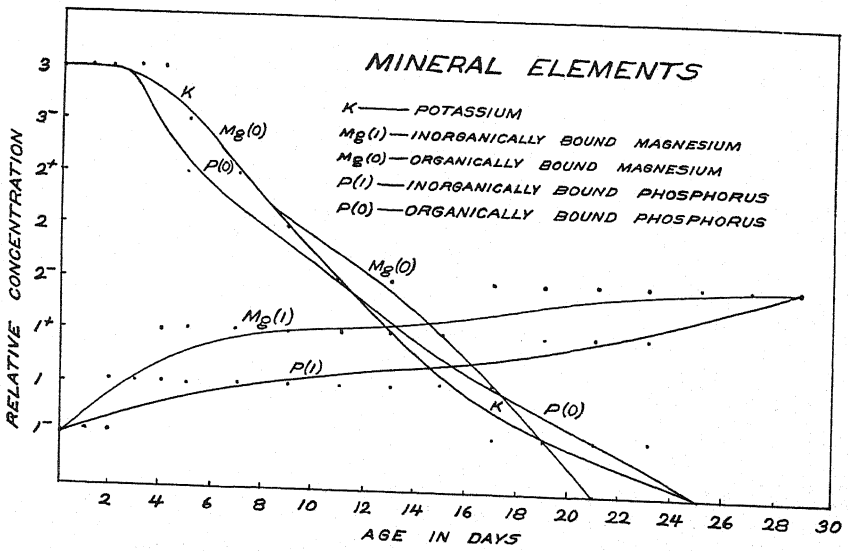
the eleventh day to a light pink on the twenty-ninth day. No asparagine was found in the cotyledons.

It was not until the second day that any noticeable change in the oil

occurred and then there was only a slight decrease in the oil content at the base of the cotyledons. It was difficult to determine the decrease in the oil content of the cotyledons the first few days of germination because of



TEXT FIG. 3. A graphic representation of the changes of oil content in the cotyledons.



TEXT FIG. 4. A graphic representation of the changes of mineral element content in the cotyledons.

the abundance of oil present. By the fourth day there was a very marked decrease of the oil contents in all parts of the cotyledons. On the ninth

day only the palisade contained more than traces of oil, and by the twenty-fifth day the oil content of this tissue had been reduced to traces.

Minerals. There was a slight increase during the first days in the amount of inorganically bound phosphorus and magnesium, and a small decrease of potassium and of organically bound phosphorus and magnesium. The potassium decreased first from the base of the cotyledon. A negative test was obtained for organically bound magnesium on the twenty-first day, and for potassium and organically bound phosphorus on the twenty-fifth day. The inorganically bound phosphorus and magnesium gradually increased in the cotyledons during germination and gave fair tests on the last day.

Plumule

Carbohydrates. Starch appeared in the base, and to some extent in the leaves, of the plumule during the first day in the germinator. It continued to increase as germination progressed and by the fifth day it was abundant in most parts of the plumule. The greatest localization in the leaves was in the tissues surrounding the veins. All of the non-reducing sugar had disappeared by the fourth day. At no time during the five days was a test obtained for reducing sugar.

Proteins and Oils. The color intensity of the biuret reaction changed from dark purple to purple on the third day and to a light purple the fifth day. The growing point of the plumule always gave a dark purple reaction. The Millon reaction was always dark red. Asparagine did not appear in the plumule during the five days it was studied. The oil gradually disappeared after the first day, and only a small amount remained on the fifth day.

Minerals. The mineral content of the plumule changed only a small amount during the five days. The potassium, inorganically bound magnesium, and organically bound magnesium decreased slightly, while both forms of phosphorus remained about the same.

Epicotyl, Hypocotyl, and Root

Carbohydrates. During the first twenty-four hours of germination starch appeared in the root and root cap, and increased to some extent in the hypocotyl. At the same time reducing sugar appeared in the hypocotyl, while the non-reducing sugar disappeared from the base of the hypocotyl. By the third day the starch and reducing sugar had reached their maximum concentration in all parts of the seedling. The greatest localization of starch was at the apex of the hypocotyl, and it gradually decreased toward the root tip, while the sugar occurred in greatest abundance in the base of both the hypocotyl and root, and decreased very rapidly toward both the root tip and cotyledons. No reducing sugar was found at the root tip (see series *D* and *E* of Pl. VI). The non-reducing sugar had all disappeared from the hypocotyl by the third day. After the third day there was a

gradual decrease in the starch and reducing sugar. No starch remained in the base of the root by the seventh day, and only a trace of sugar which disappeared during the next two days. The starch always disappeared from the cortex and pith before it did from the starch sheath. As the seedlings developed there was a gradual depletion of carbohydrates, which progressed up the seedlings, the starch disappearing about two days before the sugar. By the fifteenth day all starch disappeared and by the seventeenth day no sugar was detected.

Protein and Oil. A change in the intensity of the biuret and Millon reactions occurred in the base of the hypocotyl and root on the second day. The apex of the hypocotyl did not show any change until the fourth day and then only in the biuret reaction. The root tip always gave a dark purple color for the biuret reaction and a dark red for the Millon which was probably due to a large amount of assimilated protein in the meristematic cells. Asparagine appeared in the base of the hypocotyl on the third day, and in the apex of the hypocotyl the following day. As the seedlings became older asparagine accumulated in the base of the root, the hypocotyl, and in the base of the epicotyl, but it was not found at any time in the apex of the epicotyl. At the same time the protein reactions gradually decreased in intensity except at the apex of the epicotyl where they remained constant and always gave fairly strong tests.

The oil content decreased quite rapidly in all parts of the seedling during germination. It had disappeared from the apex of the root by the third day, from the base of the root, the hypocotyl, and the base of the epicotyl by the fifteenth day, and from the apex of the epicotyl the seventeenth day.

Minerals. During the first five days there was an increase in the amount of inorganically bound phosphorus and magnesium in the hypocotyl and root. The organically bound phosphorus and magnesium decreased somewhat at the apex of the hypocotyl but remained about constant at the base of the hypocotyl and root. Potassium decreased slightly after the third day in all parts, except the apex of the root where it remained in abundance. After the fifth day the mineral content of the base of the root and the hypocotyl remained about constant, except for the last few days when it was impossible to get tests for organically bound phosphorus and magnesium. A gradual decrease occurred in all mineral elements between the eleventh and twenty-ninth days at the base of the epicotyl, and of the organically bound phosphorus and magnesium at the apex.

A detailed summary of the results are given in tables 1, 2, 3, 4, 5, and 6.

To sum up briefly: the first changes evident during germination were the appearance of reducing sugar in the hypocotyl and root; starch in the root cap; and an increase of starch in the cotyledons, hypocotyl, and plumule. On the third day the starch and reducing sugar reached their maximum concentration in the hypocotyl and root. Non-reducing sugar was not detected in the hypocotyl, plumule, and cotyledons after the third, fourth,

TABLE 1. *The Distribution and the Relative Concentration of Organic Substances in the Different Parts of the Soybean Embryo from the Beginning of Germination to the Fifth Day*

Age in days	Plumule					Cotyledons										Hypocotyl										Root					
						Base					Palisade					Other Tissues					Apex					Base					Apex	
	Oil	Starch	Reducing sugar	Non-reducing sugar	Asparagine	Oil	Starch	Reducing sugar	Non-reducing sugar	Asparagine	Oil	Starch	Reducing sugar	Non-reducing sugar	Asparagine	Oil	Starch	Reducing sugar	Non-reducing sugar	Asparagine	Oil	Starch	Reducing sugar	Non-reducing sugar	Asparagine	Oil	Starch	Reducing sugar	Non-reducing sugar	Asparagine	Oil	Starch	Reducing sugar	Non-reducing sugar	Asparagine
0	2	0	0	1+	0	3	1−	0	3	0	3	1	0	3	0	2+	0	0	2	0	3−	1	0	2	0	2	0	0	0	0	0	0	0	0
1	2	1	0	1+	0	3	1−	0	2+	0	3	1+	0	3	0	2+	1−	0	2	0	1+	1	0	1−	0	1	0	0	0	0	0	0	0	0
2	2	1−	0	1	0	2+	1	0	2	0	3	2	0	3−	0	2	1	0	2−	0	2	1	0	1+	0	1	0	0	1−	0	0	0	0	0
3	1+	2−	0	1−	0	1+	1+	0	1+	0	3	2+	0	2	0	2	1+	0	1+	0	2	2	0	1+	0	1	0	0	1−	0	0	0	0	0
4	1	2	0	0	0	1	2	1−	1−	0	2+	3	0	2−	0	1+	2	0	1	0	2	3	0	1	0	2−	1−	2	2	0	0	0	0	0
5	1−	2+	0	0	0	1−	2+	1−	0	0	2	3	0	1+	0	1−	2	0	1−	0	1+	3	2	1−	0	2−	1−	1	2	0	0	0	0	0

0, no test obtained; 1, a trace; 2, an intermediate amount; 3, an abundance.

TABLE 2. *The Distribution and the Relative Concentration of Organic Substances in the Different Parts of the Soybean Seedling from the Seventh to the Twenty-ninth Day*

Age in days	First Internode of Epicotyl										Cotyledons								Hypocotyl Apex					Root Base			
	Apex					Base					Base		Palisade		Other tissues				Starch					Starch			
	Starch					Starch					Starch	Oil	Starch	Oil	Starch	Oil	Reducing sugar	Asparagine	Cortex & pith	Starch sheath	Oil	Reducing sugar	Asparagine	Starch	Oil	Reducing sugar	Asparagine
	Cortex & pith	Starch sheath	Oil	Reducing sugar	Asparagine	Cortex & pith	Starch sheath	Oil	Reducing sugar	Asparagine																	
7	—	—	—	—	—	—	—	—	—	—	2	1 —	3	2	2	1	0	0	1 +	3	1	1 —	1 +	0	1 —	1 —	1 +
9	1 +	3	1 —	2	0	—	—	—	—	—	1 +	1 —	3	2	2 —	1 —	0	0	1	2	1 —	1 —	2	0	1 —	0	2
11	1 —	2	1 —	1 +	0	0	1	1 —	1	1	1	1 —	2 +	2 —	1	1 —	0	0	0	1	1 —	0	3	0	1 —	0	2
13	0	1	1 —	1	0	0	0	1 —	0	1 +	1 —	1 —	2	1 +	1 —	1 —	0	0	0	1	1 —	0	3	0	1 —	0	2
15	0	0	1 —	1 —	0	0	0	0	0	2	0	1 —	1	1 +	0	1 —	0	0	0	0	0	0	3	0	0	0	2
17	0	0	0	0	0	0	0	0	0	2	0	1 —	1 —	1	0	1 —	0	0	0	0	0	0	3	0	0	0	2
19	0	0	0	0	0	0	0	0	0	2	0	1 —	0	1	0	1 —	0	0	0	0	0	0	3	0	0	0	2
21	0	0	0	0	0	0	0	0	0	2	0	1 —	0	1	0	1 —	0	0	0	0	0	0	3	0	0	0	2
23	0	0	0	0	0	0	0	0	0	2	0	1 —	0	1 —	0	1 —	0	0	0	0	0	0	3	0	0	0	2 +
25	0	0	0	0	0	0	0	0	0	2 +	0	1 —	0	1 —	0	1 —	0	0	0	0	0	0	3	0	0	0	2 +
27	0	0	0	0	0	0	0	0	0	2 +	0	1 —	0	1 —	0	1 —	0	0	0	0	0	0	3	0	0	0	2 +
29	0	0	0	0	0	0	0	0	0	2 +	0	1 —	0	1 —	0	1 —	0	0	0	0	0	0	3	0	0	0	2 +

0, no test; 1, a trace; 2, an intermediate amount; 3, an abundance.

TABLE 3. *The Biuret and Millon Protein Reactions in the Different Parts of the Soybean Embryo from the Beginning of Germination to the Fifth Day*

Age in days	Plumule		Cotyledons		Hypocotyl				Root			
					Apex		Base		Base		Apex	
	Biuret	Millon	Biuret	Millon	Biuret	Millon	Biuret	Millon	Biuret	Millon	Biuret	Millon
0	Dark purple	Dark red	Dark purple	Dark red	Dark purple	Dark red	Dark purple	Dark red	—	—	—	—
1	Dark purple	Dark red	Dark purple	Dark red	Dark purple	Dark red	Dark purple	Dark red	Purple	Dark red	Dark purple	Dark red
2	Dark purple	Dark red	Dark purple	Dark red	Dark purple	Dark red	Blue	Red	Blue	Red	Dark purple	Dark red
3	Purple	Dark red	Purple	Dark red	Dark purple	Dark red	Blue	Red	Blue	Red	Dark purple	Dark red
4	Light purple	Dark red	Purple	Dark red	Purple	Dark red	Blue	Light red	Blue	Light red	Dark purple	Dark red
5	Light purple	Dark red	Light purple	Dark red	Purple	Dark red	Blue	Light red	Blue	Light red	Dark purple	Dark red

Dark purple and dark red reactions indicate an abundance of proteins. Blue and light pink reactions indicate a small amount of proteins.

TABLE 4. *The Biuret and Millon Protein Reactions in the Different Parts of the Soybean Seedling from the Seventh to the Twenty-ninth Day*

Age in days	First Internode of Epicotyl				Cotyledons		Hypocotyl Apex		Root Base	
	Apex		Base							
	Biuret	Millon	Biuret	Millon	Biuret	Millon	Biuret	Millon	Biuret	Millon
7					Purplish blue	Dark red	Blue	Red	Blue	Light red
9	Purple	Dark red			Purplish blue	Dark red	Blue	Red	Blue	Light red
11	Purple	Dark red	Blue	Light red	Purplish blue	Dark red	Blue	Red	Blue	Light red
13	Purple	Dark red	Blue	Light red	Purplish blue	Red	Blue	Light red	Light blue	Light red
15	Purple	Dark red	Blue	Light red	Purplish blue	Red	Blue	Light red	Light blue	Pink
17	Purple	Dark red	Blue	Light red	Purplish blue	Red	Blue	Light red	Light blue	Light pink
19	Purple	Red	Blue	Light red	Light purplish blue	Light red	Blue	Light red	Light blue	Light pink
21	Purple	Red	Blue	Pink	Light purplish blue	Light red	Blue	Light red	Light blue	Light pink
23	Purple	Red	Blue	Pink	Light purplish blue	Light red	Blue	Light red	Light blue	Light pink
25	Purple	Red	Blue	Pink	Light purplish blue	Pink	Blue	Very light red	Light blue	Light pink
27	Purple	Red	Blue	Light pink	Light purplish blue	Light pink	Blue	Very light red	Light blue	Light pink
29	Purple	Red	Blue	Light pink	Light purplish blue	Light pink	Blue	Very light red	Light blue	Light pink

Dark purple and dark red reactions indicate an abundance of proteins. Blue and light pink reactions indicate a small amount of proteins.

TABLE 5. *The Distribution, and the Relative Concentration of Mineral Elements in the Different Parts of the Soybean Embryo from the Beginning of Germination to the Fifth Day*

Age in days	Plumule					Cotyledons					Hypocotyl										Root									
											Apex					Base					Base					Apex				
	K	P (org.)	P (inorg.)	Mg (org.)	Mg (inorg.)	K	P (org.)	P (inorg.)	Mg (org.)	Mg (inorg.)	K	P (org.)	P (inorg.)	Mg (org.)	Mg (inorg.)	K	P (org.)	P (inorg.)	Mg (org.)	Mg (inorg.)	K	P (org.)	P (inorg.)	Mg (org.)	Mg (inorg.)	K	P (org.)	P (inorg.)	Mg (org.)	Mg (inorg.)
0	2	1+	3	1	2	3	3	1−	3	1−	3	2	1	2	2	3	1+	1	1	2−	—	—	—	—	—	—	—	—	—	—
1	2+	1+	3	1	2	3	3	1−	3	1−	3	1+	1+	2	2	3	1	1+	1	2−	3	1+	1	1	2	3	2+	1	1−	2
2	2+	1+	3	1+	2	3	3	1−	3	1	3	1	2	2−	2	3	1	2	1	2	3	1+	1+	1	2	3	2+	1+	1−	2+
3	2+	1+	3	2−	2+	3	3	1	3	1	3	1	2	1+	2	3−	1	2+	1	2+	3	1	2	1−	2+	3	2	2	1−	3
4	2+	1+	3	2−	2+	3	3	1	3	1+	2+	1	2	1	2	2+	1	3	1	3−	2+	1	2+	1−	3	3	2	2+	1−	3
5	2+	1+	3	2−	3	3−	2+	1	3−	1+	2	1	2	1	2−	2	1	3	1	3−	2+	1	3	1−	3	3	2	3	1−	3

0, no test; 1, a trace; 2, an intermediate amount; 3, an abundance.

Mg (org.), organically bound magnesium; Mg (inorg.), inorganically bound magnesium. Similar abbreviations are used for phosphorus compounds. K, potassium.

TABLE 6. *The Distribution and the Relative Concentration of Mineral Elements in the Different Parts of the Soybean Seedling from the Seventh to the Twenty-ninth Day*

Age in days	First Internode of Epicotyl										Cotyledons					Hypocotyl Base					Root Base				
	Apex					Base																			
	K	P (org.)	P (inorg.)	Mg (org.)	Mg (inorg.)	K	P (org.)	P (inorg.)	Mg (org.)	Mg (inorg.)	K	P (org.)	P (inorg.)	Mg (org.)	Mg (inorg.)	K	P (org.)	P (inorg.)	Mg (org.)	Mg (inorg.)	K	P (org.)	P (inorg.)	Mg (org.)	Mg (inorg.)
7	—	—	—	—	—	—	—	—	—	—	2+	—	1	—	1+	2	—	2+	—	2+	2	—	2	—	2
9	3	1+	3	1+	2+	—	—	—	—	—	2	2	1	2	1+	2	1	2	1	2	2	1	2	1	2
11	3	—	3	—	3	3	—	3	—	2+	2−	—	1	—	1+	2	—	2	—	2	2	—	2	—	2
13	3	1+	3	1+	3	3	1	2+	1	2+	1+	1+	1	2−	1+	2	1	2	1	2	2	1	2	1−	2
15	3	—	3	—	3	2+	—	2+	—	2+	1	—	1	—	1+	2	—	2	—	2	2	—	2	—	2
17	3	1+	3	1+	3	2+	1	2+	1	2+	1−	1	1	1	2−	2	1−	2	1−	2	2	1	2	1−	2
19	3	—	3	—	3	2+	—	2+	—	2+	1−	—	1+	—	2−	2	—	2	—	2	2	—	2	—	2
21	3	1+	3	1	3	2+	1−	2+	1−	2+	1−	1−	1+	0	2−	2	1−	2	1−	2	2	1	2	1−	2
23	3	—	3	—	3	2	—	2+	—	2	1−	—	1+	—	2−	2	—	2	—	2	2	—	2	—	2
25	3	1+	3	1−	3	2	1−	2	1−	2	0	0	2−	0	2−	2	1−	2	1−	2	2	1	2	0	2
27	3	—	3	—	3	2	—	2	—	2	0	—	2−	—	2−	2	—	2	—	2	2	—	2	—	2
29	3	1+	3	1−	3	2	1−	2	0	2	0	0	2−	0	2−	2	0	2	0	2	2	0	2	0	2

0, no test; 1, a trace; 2, an intermediate amount; 3, an abundance.

Mg (org.), organically bound magnesium; Mg (inorg.), inorganically bound magnesium. Similar abbreviations are used for phosphorus compounds. K, potassium.

and seventh days, respectively. Starch was not detected in the root, hypocotyl, epicotyl, and cotyledons after the seventh, thirteenth, fifteenth, and nineteenth days, respectively. Reducing sugar disappeared in the same order, but about two days after the starch, except in the case of the cotyledons where only a possible trace was found on several days. A marked decrease in the amount of oil in the cotyledons occurred by the fourth day; depletion began at the base of cotyledons and progressed toward the opposite end; the palisade was depleted last. The biuret protein reaction in the cotyledons changed from a dark purple to a purplish blue during the first seven days of germination, while the Millon reaction showed no marked change until the thirteenth day. All growing points always gave intense protein reactions. Asparagine appeared in the hypocotyl on the third day. By the twenty-ninth day it was abundant in the hypocotyl and the base of the epicotyl and root. It was not detected in cotyledons. Both the organically bound phosphorus and magnesium in the cotyledons gradually change to the inorganic forms, a considerable portion of which remains in the cotyledons. On the other hand most of the potassium disappears from the cotyledons.

During the first five days of germination when tests were made on all parts of the root, inorganically bound phosphorus, oil, starch, reducing sugar, and asparagine were not detected in the meristematic tissue of the root tip. This may be of interest in regard to the hypothesis proposed to explain rhythm in the growth of root tips, but more data at intervals following closely the growth rhythm are necessary to determine what bearing the different foods and mineral elements present might have upon the rhythm. The root tip always gave strong protein reactions; a strong test for an oily substance not soluble in acetone, probably lecithin; a fair test for organically bound phosphorus, and a strong test for potassium.

Discussion

Carbohydrates

Street and Bailey (20) reported 3.31 percent of sucrose in the Hollybrook variety of soybeans. The non-reducing sugar found in the cotyledons and hypocotyl of Manchu soybeans is no doubt sucrose too, as it is hydrolyzed by invertase to reducing sugars that readily form osazones *in situ* in sections of the cotyledons. This sugar may be the source of much of the reducing sugar which appeared in the hypocotyl and root during the first five days of germination. After this non-reducing sugar is used it was not determined what was the direct source of the reducing sugar. Sachs was of the opinion that in some oily seeds all of the oil was changed directly into starch, while in other seeds only part of it was changed to starch, the rest being converted directly into sugar. Miller stated that the marked increase in the amount of sugar during the progress of development of sunflower seedlings

makes it certain that it has its origin in the oily reserve. While this conclusion has been universally accepted, since the time of Sachs, the intermediate products remain to be definitely determined. In the epicotyl, hypocotyl, and root of the seedling the reducing sugar always disappeared within two days after the depletion of starch, even though oil was present. It was not determined whether a similar relationship exists in the cotyledons, for only on a few occasions was a test obtained that would indicate the presence of reducing sugar in the cotyledons. Miller did find a small amount of reducing sugar in the cotyledons of germinating sunflower seeds. It was thought that the reducing sugar might be diffusing out of the cotyledons as fast as it was formed but when the cotyledons of a number of seedlings were pinched off, and tested at the end of twelve and twenty-four hours, no sugar had accumulated. The sugar if formed may have been oxidized in respiration or converted to some other substance, or both, as rapidly as it was formed.

The starch content of soybeans varies greatly, even in the same variety, as has been shown by Stark (19). Muramatsu (11) analyzed the ripe and unripe seeds of several varieties of soybeans for starch and obtained the following percentages.

Variety	Unripened Seeds	Ripened Seeds
Yagi	4.78%	.019%
Niciwase	2.60%	.010%

These figures show that as the seeds ripened a large amount of the starch was changed to some other substance. As the seeds germinate a reverse process occurs, for a large amount of starch appears in the cotyledons, especially in the palisade. Why the palisade plays such an important part in the transformation of organic substances is not known. During the first few days of germination carbohydrates appear in the hypocotyl and root more rapidly than they are used. The starch accumulates largely in the least active tissue, the reducing sugar in tissue that is somewhat more active, while neither accumulates at the growing point. This is probably due to the fact that the sugar is utilized in the formation of new cells as rapidly as it arrives at the growing point.

Oils

The fact has been well established by a large number of workers that at least part of the oil in fatty seeds is broken down into free fatty acid and glycerine during germination. The idea of Schmidt (16) that oil is transported as such in the plant has been questioned by Rhine (14). Miller was not able to find more than a small amount of free acid in the cotyledons of sunflower seedlings until they were well developed. Thus either the fatty acid must change very rapidly or a large portion of the oil is changed directly to some other compound. Pirschle (13) has found considerable amounts of acetaldehyde in germinating fatty seeds, which he thinks is either the normal intermediate product of the respiration of fats, or of the trans-

formation of fats to carbohydrates. The investigation of Matthes (8) with germinating sunflower seeds, shows that the oil is saponified and that the fatty acids become dispersed into middle, high, and low fatty acids. He suggests that from these originate oxy-acids, and possibly oxy-aldehydes which are built up into sugar. At the present time the exact intermediate products of oil in its transformation to carbohydrates and in oxidation is not known. It has been shown, however, by Terroine, Trautmann, and Bonnet (21) that 73 percent of the energy yield of the fat consumed in germinating fatty seeds is utilized in growth.

Proteins

The protein work was limited to general protein tests and the asparagine test, because of the lack of good microchemical tests for products of protein hydrolysis. As the protein reserves disappear from the cotyledons a large amount of asparagine appears in the hypocotyl, root, and epicotyl. Jodidi was of the opinion that the acid-amides were formed from the amino acids which in turn were formed from the polypeptides due to the degradation of proteins. Schulze (17) who worked with *Lupinus lutens* seedlings found the asparagine formed would account for sixty percent of the loss of protein nitrogen, and Chibnall (3) suggested that asparagine has the rôle of a translocatory substance and is the chief medium whereby nitrogen can be conveyed from one part of the plant to another. Some of the protein reserve must also be transported as amino acids for Pettibone and Kennedy (12) were able to detect them in the flowing sap of corn seedlings. The fact that asparagine was not found in the root during germination or in the apex of the epicotyl of the older seedlings, shows that it must be assimilated as rapidly as it diffused into these regions.

The accumulation of asparagine in the different parts of the developing etiolated seedlings is a gradual process and does not appear to have any direct relation to the period of greatest growth. Stark (18) found no correlation between protein hydrolysis and growth in developing etiolated soybean seedlings. He also states that there is a marked fluctuation in the alpha-amino nitrogen content of the seedlings. In the present study the greatest changes in the general protein reactions occurred between the third and thirteenth days when the seedlings made nearly all of their growth.

Minerals

Klein considers that most of the phosphorus in the cotyledons of *Phaseolus multiflorus* is organically bound, and that as the seeds germinate and the seedlings develop, there is a continual change of the organically bound phosphorus to the inorganically bound form which moves to the growing points where it again becomes organically bound. Similar results were obtained with soybeans. As the seeds germinated, and as the seedlings developed, both the organically bound phosphorus and magnesium in the cotyledons gradually changed to the inorganically bound form.

Inorganically bound phosphorus was abundant in most parts of the root, except at the root tip, where it was not detected. Perhaps it was utilized as rapidly as it arrived. However, both potassium and organically bound phosphorus were abundant in the root tip.

It was not determined in what chemical compounds organically bound phosphorus occurred in the ungerminated seed. Webster (23) found that 0.495 percent of phosphorus in Manchu soybeans occurs in the form of phosphatides, but this accounts for only a small portion of the phosphorus contained in the seeds.

In the exhausted cotyledons no organically bound phosphorus or magnesium was detected, but the inorganic salts of these elements were still present. Buckner (1) working with garden bean seedlings found that 46.66 percent of the minerals remained in the exhausted cotyledons.

Potassium, which according to Borst (2) is the most abundant mineral element in the cotyledons, diffused out so rapidly that no trace of it was found after the twenty-third day. This element in the seed and seedling is apparently in a water soluble form, since it may be so completely removed by washing sections of the tissue in water that no trace of it may be detected, even though the sections are ashed.

Summary

1. The cotyledons of mature Manchu soybean seeds contain a large amount of protein, oil, some non-reducing sugar, and a small amount of starch. Transformation and translocation of these reserve foods were followed microchemically during the germination and growth of soybean seedlings in darkness.

2. During germination the first changes found were the appearance of reducing sugar, an increase of starch in the hypocotyl, appearance of starch in the root cap, and an increase of starch in the cotyledons.

3. During the first three days of germination there was a large accumulation of starch in the apex of the hypocotyl, and reducing sugar in the base of the hypocotyl and root.

4. The amount of starch in the cotyledons increased until the fifth day and then remained nearly constant until the ninth day, after which there was a rapid decrease. It disappeared from the palisade last.

5. The starch disappeared from the different parts of the seedling in the following order: root, hypocotyl, epicotyl, and cotyledons. Reducing sugar disappeared from the seedling in the same order, but two days later, except in the cotyledons where only a slight test for it was obtained on several occasions.

6. Non-reducing sugar was not detected in the hypocotyl, plumule, and cotyledons after the third, fourth, and seventh days respectively.

7. Asparagine appeared in the hypocotyl on the third day. It gradually increased during the development of the seedling, until on the twenty-

ninth day it was abundant in the hypocotyl, and fair amounts occurred in the base of the root and epicotyl. Asparagine was not detected in the plumule or cotyledons or at the root tip.

8. The depletion of the oil of the cotyledons began at the base and progressed toward the opposite end. The palisade was depleted much more slowly than were the other tissues in the cotyledons.

9. There was a gradual change of the organically bound phosphorus and magnesium of the cotyledons to the inorganically bound. Although both inorganically bound phosphorus and magnesium moved quite rapidly toward the growing points, they gradually increased in amount in the cotyledons.

10. Potassium moved out of the cotyledons more rapidly than did the phosphorus and magnesium, and by the twenty-third day not enough remained to give a test. Platinum chlorid failed to give a positive test for potassium on fresh sections, but a copious precipitate of potassium chloroplatinate crystals was obtained on ashed sections. When sections of the tissue were washed for two days a test for potassium was not obtained even though the sections were ashed. Apparently the potassium in the sections is water soluble.

11. Starch, reducing sugar, asparagine, oil and inorganically bound phosphorus were not detected in the meristematic tissue of the root tip.

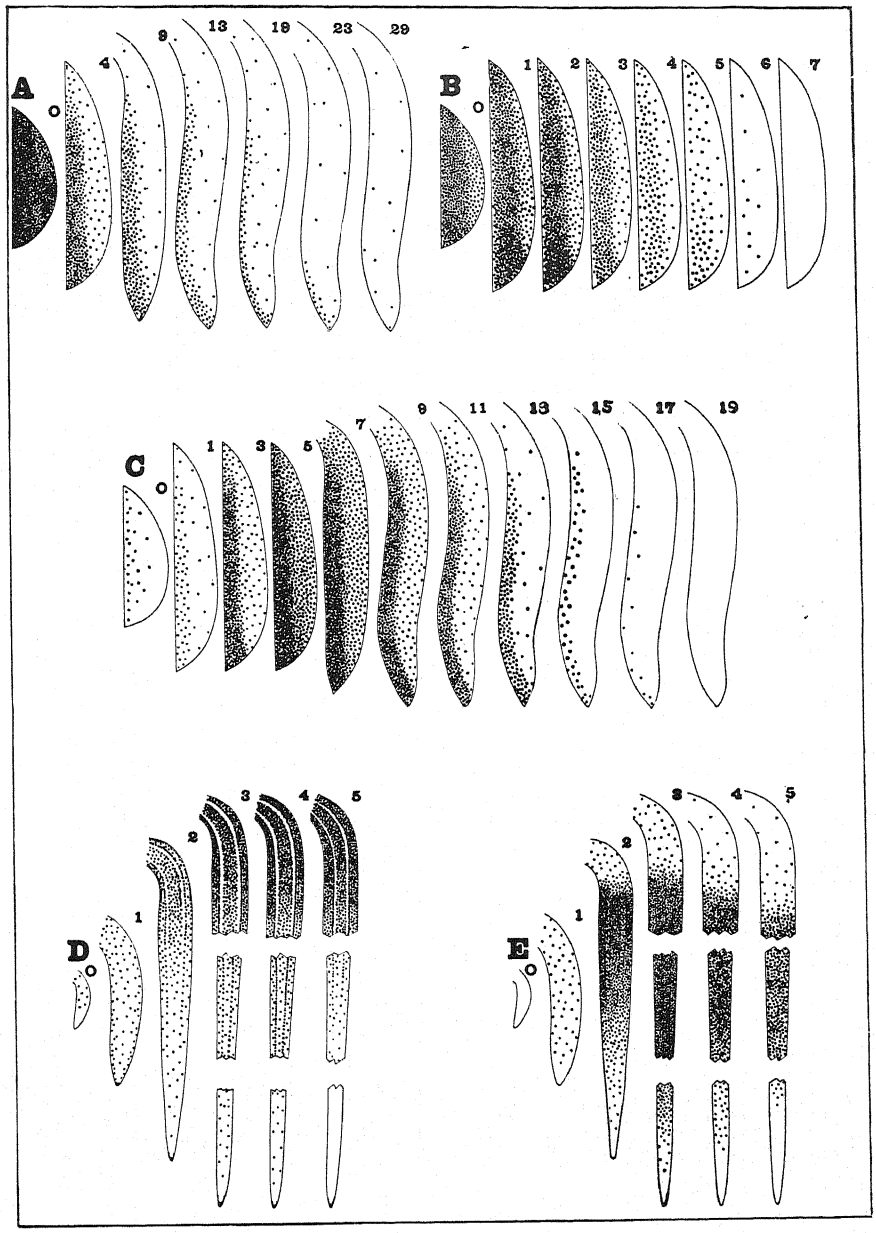
12. There is need for a detailed analysis of the intermediate products that occur as the oil in germinating seeds breaks down and other organic substances such as sugar and starch are formed.

The writer wishes to express his appreciation to Dr. H. C. Sampson under whose direction this study was made, and to Dr. R. C. Burrell who made many valuable suggestions in regard to various parts of the work.

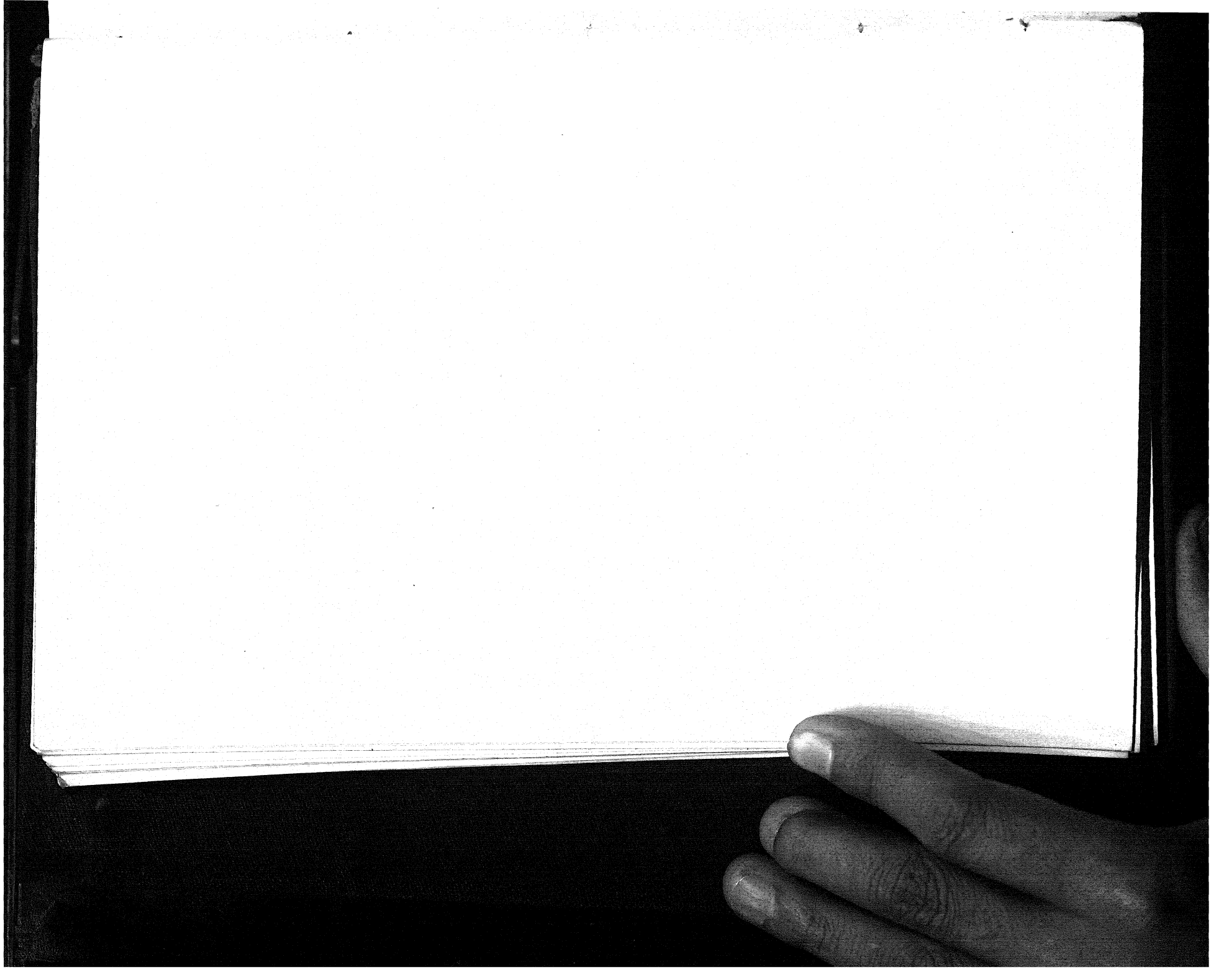
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EXPLANATION OF PLATE VI

The plate illustrates diagramatically the relative distribution of organic substances in the seed and seedling. All of the series are longitudinal sections, the first three are of the cotyledons and the last two are of the hypocotyl and root, respectively.

Series *A* shows the localization and the depletion of oil in the cotyledons from the beginning of germination to the twenty-ninth day.

Series *B* represents the localization and depletion of non-reducing sugar in the cotyledons from the beginning of germination to the seventh day.

Series *C* shows the localization, increase, and decrease of starch in the cotyledons from the beginning of germination until the nineteenth day.

Series *D* shows the distribution and relative concentration of starch on different days in the hypocotyl and root from the beginning of germination to the fifth day.

Series *E* shows the distribution and relative concentration of reducing sugar on different days in the hypocotyl and root from the beginning of germination to the fifth day.

THE REACTIONS OF *CLAVICEPS PURPUREA* TO VARIATIONS OF ENVIRONMENT[1]

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INTRODUCTION

Claviceps purpurea (Fr.) Tulasne is an outstanding species among parasitic fungi. Forming its conspicuous sclerotial stage in the heads of rye and other grains, it very early came under observation. Long before its developmental history was known, it was looked upon with suspicion, and later was proved to be the cause of disastrous epidemics of gangrenous disease.

In its sclerotial stage, *C. purpurea* elaborates a violent poison which later, mixed with grain or flour, attacks human beings and animals alike. Reference to epidemics of this dreadful affliction are found in the literature as far back as the time of Caesar, and outbreaks of the much-feared "sacre feu" were frequent down through the centuries among the European peasantry. People gradually came to associate the terrible scourge with the years when an intensely cold winter was followed by a very rainy summer, conditions that favor the development of this fungus.

Claviceps purpurea passes through a most interesting cycle of growth. The horny, dark-violet sclerotia fall to the ground when the rye head is mature. In the spring those which survive the winter send up stromata whose small, globose heads later contain the perithecia. The asci normally ripen as the new rye crop comes into bloom. Thread-like ascospores are ejected in great numbers, and are borne, chiefly by insects, to the open flowers. By infection of the ovary, further normal development of the rye kernel is prevented. Instead there ensues abundant conidial (sphacelial) formation. A sweet liquid, "honey dew," heavily laden with the conidia, forms during this early stage and exudes from the flowers in drops or even in tiny streams. After this period of vigorous conidial fructification, the mycelial mass so formed gradually changes into the dry, hard, violet to blackish resting-stage, in which form it may retain vitality for at least a year.

Not until the eighteenth century was the full life history studied. Tulasne (38) first followed the fungus through its development under natural conditions, and his work was later confirmed by Brefeld (7), Lindau (22), and other observers. Brefeld was the first to attempt controlled

[1] Papers from the Department of Botany of the University of Michigan, No. 330.

culture of *C. purpurea* upon laboratory media. As for so many other fungi, he used moist bread as a substratum upon which to sow the ascospores. He describes rather briefly the conidial (sphacelial) growth which ensued in the cultures. His work was confirmed by Engelke (11) who grew the conidial stage upon both liquid and solid media. Like Brefeld, he started with the ascospore stage, and his resulting cultures were wholly of conidial type.

Meyer (25) also grew *C. purpurea* in pure culture, but he planted conidia from the honey dew, instead of ascospores. His results are in agreement with those of the preceding workers, but he gives a considerably more detailed description of the organism in culture. He used a most complex liquid medium in an attempt to duplicate the chemical composition of young rye heads, but did not succeed in stimulating sclerotial development.

Stäger's work on *C. purpurea* (30, 31, 32, 33, 34, and 35) was confined mainly to its biology and distribution. He champions the hypothesis of "biological races" of this species, *e.g.*, on *Lolium*. However, he also grew the organism in culture and found that the conidia, like the sclerotia, may remain viable for at least a year, a fact later confirmed by Bonns (6), and many times found to be true in the experiments of the writer, who has had conidial cultures live four and one-half years from the time of planting.

McFarland (24) likewise carried on field-inoculation studies, in the course of which he isolated twelve "strains" which he grew on numerous media. He criticizes Stäger's work adversely, and believes Stäger announced the existence of a biological race on *Lolium* without sufficient data. McFarland found no evidence that such biological races occurred upon cereals and common grasses. He rightly stressed the necessary experimental condition that *glumes must be open* in the host plant if either natural or controlled infection is to occur.

To Vincens (39) and to Killian (16) we owe such knowledge as we have of the sexual development by means of antheridial and ascogonial structures, but further cytological work needs still to be done.

The sclerotial stage, commonly designated "ergot," occupies an important place among therapeutic agents, so it has been the subject of considerable investigation with a view to utilizing its commercial value. Professor Ludwig Hecke (14, 15), in his experiments on inoculating blooming rye in the field, reached the conclusion that the fungus could be grown on an industrial scale with reasonable profit. Quite recently, Bonns (6) made an attempt to culture this organism in the laboratory because of its commercial importance, but he failed to secure sclerotia and found that the conidium-bearing mycelium was practically lacking in medical value.

That no previous worker had achieved the goal of securing sclerotia in artificial cultures indicated that the problem presented unusual difficulty. Nevertheless, a review of these various contributions clearly showed the

desirability of a thorough physiological study under carefully controlled conditions, of course with the hope of determining the essential factors for production of sclerotia provided by the living host. Such a study has been undertaken by the writer during the past several years, with the results herein reported.

Technique

Isolations. In the beginning, isolations were made from sclerotia obtained from many regions: Germany, Russia, Portugal, Spain, Holland, and France, as well as several localities in the United States.

In saprophytic culture, these strains were found to be closely similar when conditions were identical. Three were chosen for comparative study under various nutritional conditions. After more than a year of study, when it had become evident that there was no significant difference among them, the work was limited for convenience to a single culture which had been isolated by the writer in 1922 from Spanish ergot of high quality. From time to time new isolations have been made from fresh sclerotia, with which this culture has been carefully compared. In 1926, after it had been saprophytically maintained for more than three years, it was tested on rye (*Secale cereale* (L.) Beauv.) and on quack grass (*Agropyron repens* L.) for any sign of diminished parasitism. Hecke's technique (14, 15) was used in this outdoor work as far as possible, but the plants were covered with parchment caps before anthesis in order to prevent or at least decrease pollination. When flowers were opening they were sprayed with a conidial suspension and recapped. The rye readily became infected, forming large, normal sclerotia, from four to thirteen per head. The quack grass, likewise, was heavily infected with typical sclerotia. It is felt, therefore, that the culture has maintained full virility during the time of experimentation.

In the laboratory, cultures were grown in glass containers of common types: large test tubes (35 × 240 mm.); capsules of from 25 cc. to 250 cc. capacity; liter Erlenmeyer flasks, so slanted as to give a large surface and abundant supply of food and moisture. Parent strains were isolated and maintained under room conditions upon Thaxter's hard potato agar. Upon this substratum they produced only a thin mycelial mat, with numerous small conidia over the entire surface. If they are properly transferred, not more than six times per year, no degeneracy is to be noted. Cultures maintain full character on this base for an indefinite time, and are readily transferable to such other media and conditions as may be chosen for investigation.

In general, planting was done by means of a conidial suspension, prepared from stock cultures not less than a month old to ensure maturity of the growth. Frequently, however, for the purpose of comparison or merely for convenience, tissue transplants were made directly to the surface of the new medium. Ensuing growth was the same whichever method was used. Suitable control cultures for all of the experiments were planted upon

either potato agar or Leonian's medium (20, 21, modified as will be noted later), or upon both of these, as seemed advisable for the purpose in view.

Nutritional Studies. Preliminary study showed that many cereal agars and mashes offer acceptable substrata for good growth of *Claviceps purpurea.* As shown in table I, nearly all of those media tested permit good vegetative development. Cultures retain vitality over a long period of time, but the author has never obtained, either in cereal mashes or in cereal agars, any semblance of such a cortex layer as was reported by Bonns (6), although it has occurred upon various other media as will appear later. In the mashes, growth penetrated throughout the whole substance, not just over the surface. Moreover, on the heavy cereal agars, where the mat of growth often became deeply wrinkled as the food supply was depleted, the convolutions remained uniformly tender throughout the long life of the culture, except only in the little knots of denser growth herein discussed as *pseudosclerotia* (p. 56).

Oatmeal, rye meal, cornmeal, soy bean meal, ground rye, and ground wheat were used (*a*) in the form of mashes and (*b*) in solid form by the addition of agar. Except for the fact that in the mashes the penetration of mycelium occurred throughout the whole substance, growth was not different from that obtained upon the corresponding solid medium. Hence it is felt that one tabulation may well suffice for both series of results.

Several "synthetic media" were also tested for comparison with cereals: the formulae of Engelke (11), of Coons (8), and of Leonian (20, 21). As table 1 shows, only Leonian's proved equal to the cereals for promotion of growth.

Meanwhile a more closely controlled study was under way in a search for readily available sources of carbon. For each series, control cultures were grown upon Leonian's agar medium for comparative purposes. In testing the various sugars as a source of carbon, the following basic formula was used both in liquid form and as an agar medium, sugar being added in varying amounts:

Calcium nitrate	1.0 g.
Dihydrogen potassium phosphate	1.25 g.
Magnesium sulfate	0.625 g.
Water	1000 cc.
Test sugar	1, 2, 3, 5, 8, and 10 percent

Maltose, dextrose, levulose, and sucrose were tested in the above amounts, final judgment being based upon not less than ten cultures for each percentage. Cultures were allowed to run until a static condition was reached, usually about five weeks, before final records were made. The higher content of sugar did not prove to be favorable in any case. Growth was decidedly better at 2 percent and 3 percent than at 1 percent, but it was no better at 5 percent or 8 percent than at 3 percent. At 10 percent, cultures did not progress much beyond germination. Even where some initial

growth occurred, the cultures soon lost vigor, and final development was below that of all the other cultures, even those at 1 percent.

TABLE 1. *Comparative Growth of Claviceps purpurea upon Various Media* *

Medium	Mycelial Development	Conidial Development	Further Development
Hard potato agar	I	I	Lacking
Oatmeal	III	III	Numerous pseudosclerotia formed on drying
Cornmeal	III	III	As above
Rye meal	III	III	As above
Soja bean meal	I	I	Lacking
Ground rye	III	III	As on oatmeal
Ground wheat	III	III	As on oatmeal
Engelke's medium	I	I	Lacking
Coons's medium	I	0 to I	Lacking
Leonian's medium	III	III	As on oatmeal

* In the above table, relative amount of growth is indicated by the Roman numerals: I indicates the minimum, III the maximum development here obtained of the factor concerned.

Maltose proved to be decidedly the most favorable sugar of those tested for growth of this organism. Not much difference was apparent between dextrose and levulose, but sucrose appeared quite poor as a carbon source for *C. purpurea*. In none of the entire series could it be said that growth was more than half as heavy as it is upon the cereals. Since maltose is probably used by the organism only after digestion to dextrose, it perhaps may be assumed that the clear advantage of maltose over dextrose lies in the fact that digestion takes place *pari passu* with utilization. Twice as much growth could thus be insured with maltose since it provides twice as much carbon as does dextrose in a solution of the same osmotic pressure. In any case, since the superiority of maltose over the other sugars was unmistakably demonstrated, it was definitely chosen as the standard sugar for subsequent use.

Selection of a Standard Medium. Several of the more common nitrates were tested, but none of them proved to be capable of developing growth as good as that normally obtained upon peptone. Meanwhile, the growth of the organism upon Leonian's agar suggested that a modification of his formula might be of advantage in attaining the desired heavy increase of growth. This nutrient mixture was therefore tested both with and without the malt extract ingredient which Leonian found so useful in his studies. While it proved to be very satisfactory for vegetative growth and conidial formation, better results were secured when the malt extract was retained. After much experimentation, this formula appeared to be the most promising of all those tested and later, *using double quantities of the nutrients and 6 percent agar*, it became the standard medium used in all other phases of the investigation unless otherwise stated.

Physiological Considerations

Description of Typical Culture

Previous descriptions of *C. purpurea* in saprophytic culture have been based upon growth in or on a variety of media, which probably explains the prevailing differences of opinion in regard to hyphal arrangement. In order, therefore, to have a standard for the mycelial and conidial developmental history, a thorough microscopical study was made of cultures growing upon this chosen medium. In the early stages, gelatinous growth spreads slowly in all directions from the point of inoculation. Hyphae are relatively few at this time, but within forty-eight hours conidia are abundant. They show wide variation in size, ranging from 2.2 by 1.3 to 8.5 by 3.2 microns. Daily examination of material taken from points equidistant from the original inoculum show that conidia of maximum size are produced in about fifty hours after planting, or in about twenty-four hours after conidial formation is well started. Conidia are typically one-celled, hyaline, ellipsoidal, often with bi-polar "oil-drops," as noted by Meyer (25), which are doubtless the same as the polar granular areas observed in other species, such as *C. paspali*. Germinating conidia typically show mycelial development initiated at or near these drops, but quite often the hyphae are seen to start from the lateral portions of the conidium also.

This gelatinous mass of growth soon becomes firmer by the increase of mycelial development, and the plicate area is usually 3.5 to 4.0 cm. in diameter within a week. Subsequent growth is relatively slower. If left in the dark, little or no upright hyphal development ensues, though a good horizontal mat is formed. If, however, the cultures are placed in direct or strong diffused light, they respond very quickly by producing aërial mycelium over the entire surface to a height of 1.0 to 1.5 mm., while the horizontal mat normally becomes about 1.0 mm. thick. As will appear later, this upright growth has been markedly increased in both thickness of mat and height of the hyphae when cultures were put under specially favorable conditions. No grouping of hyphal threads such as Meyer (25) described has ever been noted in the horizontal web, nor have coremium-like bundles ever been observed in the normal mycelium. The upright hyphae branch repeatedly but do not attach to each other in any way. In four to five weeks a static condition has occurred. Further development is not observable for some time, but bits of the fungus from all parts of the culture are fully viable and develop typically if placed upon fresh medium.

After a further period of several weeks, when the culture has been planted from ten to fifteen weeks, characteristic bodies begin to develop in the mat. It seems beyond doubt that these are the structures called by Engelke "microsclerotia." Unfortunately, he merely noted their occurrence, giving no description or illustration with which comparison could be made. They constitute one of the most interesting phenomena encountered,

occurring consistently in old, drying cultures. When one is teased apart or crushed under the glass, it shows a mass of closely packed hyphae, with marked anastomosis of the hyphal strands. Conidia of this growth seem especially rich in the oil drops. Except in these two points, however, such growth does not appear to differ from that of the normal mat. These knots of growth round up into pulvinate to hemispherical structures, embedded in the loose hyphae of the mycelial mat. They are usually very small, not often more than 2 mm. in diameter.

Various sizes of these structures were examined histologically, from small, forming ones to the older, dried, sclerotioid ones. They stain fairly well in haematoxylin-alum (Mayer's haemalum). In sections of the older stages, as shown in the photomicrographs (Pl. VII, figs. *a*, *b*), vacuolated areas are numerous, but in no stage could any sign of differentiation of tissues be found. In general appearance such a section is quite strongly suggestive of the inner (prosenchyma) tissue of a dried sclerotium; but the vacuolated areas are larger and more numerous than in the true sclerotial tissue, and there is nothing comparable to the pseudoparenchyma or rind portions of the natural sclerotium. It cannot, therefore, be said that these are real sclerotia, even of "micro" type, hence the writer has preferred to designate them by the term pseudosclerotia. The term *pseudosclerotium* as herein used designates a small, hemispherical, sclerotioid body, composed of dense layers of hyphae covering an inner, vacuolated tissue, and embedded in the mycelial web (Pl. VII, fig. *d*). Whether such a structure may be considered the primordium of a sclerotium that did not meet with conditions which would permit full development, the writer is not prepared to maintain. It is certain, nevertheless, that such bodies are a part of the natural development of a thrifty culture, grown saprophytically under normal conditions, and that they are affected favorably by the influences which permit the maximum development of this fungus in culture. Typically, they appear only after the culture has aged and become relatively dry, but their appearance can be hastened and their size can be increased by special treatment of the mycelial mat where they originate, as will be shown later. When such bodies are freed from clinging mycelium by dropping them into alcohol and washing in water before transferring to fresh nutrient, they readily develop mycelium and conidia just as does a bit of the tissue from the center of a true sclerotium, and subsequent growth corresponds closely to ordinary conidial cultures.

Special Stimulants

Ergot Extract. Several special stimulants were tried in the hope of inducing the excellent vegetative growth to make further development and differentiation. An aqueous extract of ergot was prepared from high grade Spanish Ergot, by the British Pharmacopoeia method. This was sterilized by passage through a clay filter (Mandler) and added to the standard medium, one part in twenty-five. The fungus on this medium developed

a heavy, firm mycelium, 1.5 to 2.5 mm. thick, with aërial hyphae 2.5 to 3.0 mm. tall, which showed a tendency to form groups or hyphal bundles quite similar to coremia. Conidia were greatly decreased in number, but those present were of typical appearance, though somewhat larger than usual. As usual, pseudosclerotia were formed as the cultures dried after maturity and were slightly larger on the average, but no further differences were found from the usual growth. Meanwhile, however, because of the noticeably heavier growth of mycelium, a variation of the experiment was made by doubling the strength of the B.P. Extractum Ergotae, and adding it to the medium one part in ten. This decidedly stimulated the rate of growth, and also shortened the period preceding formation of pseudosclerotia which now appeared in less than three weeks without the preliminary drying of the medium which heretofore had preceded them. As time passed, however, it was found that the stimulating effect was confined to the early stages of development. In three or four months, these cultures showed no further sign of differentiation than did the control cultures without the extract. The experiment was repeated several times and always the heavier, quicker development was clear-cut, but it proved to be only a matter of favorable nutrient spurring the initial growth, and not of effective stimulation to any greater final development.

Vitamin Extract. It is well known that animal reproduction is greatly influenced by the presence or absence of Vitamin E. Because of this important property, it was decided to test the effect of such an extract upon the development of *C. purpurea.* The extract is of an oily nature, and great care is necessary to incorporate it in the medium and prevent it from merely forming a thin film over the surface. It can be done successfully, however, by cautiously adding it when the medium is just ready to gel. The material was used in both crude and concentrated, or purified, form and in every case it strongly stimulated the fungus and caused an early development of the pseudosclerotia. No further development could be induced, however, even when the amount was increased from two to five and then to ten drops per twenty cc. of the medium.

Sprouted Rye. Well-filled grains of rye were selected, passed through an air blast to remove dust, washed thoroughly in tap water, dipped for one minute into 1–1000 bichlorid of mercury solution, washed several times in sterile, distilled water, and allowed to sprout, five grains being placed in each test-tube. When the sprouts were about 2 cm. long, a conidial suspension was sprayed into the tubes. The fungus grew rapidly, the very tough, heavy mat of mycelium soon checking the growth of the young shoot. This mat differed in three ways from that grown on standard medium: (*a*) it was very tough; (*b*) it developed very few conidia; (*c*) it never developed any of the pseudosclerotia so characteristic of the aging growth upon nearly all media. The method was varied by pouring cooled, nutrient agar over the sprouted rye and planting it with the fungus in the usual way.

The same tough, heavy growth was obtained as on the rye alone, but no tendency toward greater sclerotinization was ever obtained.

Physical Factors

It is recognized that in the most carefully regulated experimentation dealing with physical factors, more or less overlapping of influence is unavoidable. So far as it could be done, however, each factor was studied as a unit, keeping all other influences at their optima. The salient matters chosen for detailed study were the effects of temperature, moisture, light, pressure, transpiration, and oxidation.

Temperature

C. purpurea is able to make normal saprophytic development over a rather wide temperature range, from 18° to 28°–30° C. Hence room temperature has proved entirely suitable during the course of the work. When transplants were placed immediately in an environment below 10° C., germination occurred slowly and subsequent development was very much retarded and scanty in amount, although the organism remained alive for several months. During this time, any cultures removed to room conditions quickly revived, and growth then proceeded in normal manner. If transplants were placed at once in an oven kept at 37° C., the conidia germinated very quickly. If then removed to room conditions, typical development ensued. If left at the high temperature, however, cultures did not progress much beyond germination. At best, a thin film of distorted growth was formed which in the fourth week failed to give living subcultures. Between 10° C. and the optimum, ranging from 20° C. to 30° C., vigor and rapidity of growth are in direct ratio to the temperature.

Thrifty young cultures, started under room conditions and a week later subjected to the extremes, showed noteworthy results. Those transferred to the cold were quickly checked and made little or no subsequent growth while left in the cold but, if they were returned to the laboratory at any time within the succeeding eight weeks, the cultures again became active and made typical development. Comparable, thrifty, week-old cultures transferred from room conditions to 37° C., however, quickly showed harmful effects. Growth was checked in a few hours. Within a week the hyphal strands showed marked wilting and the general appearance of the culture became very atypical. Even when removed to room conditions, growth was not resumed in these cultures. For several weeks, however, portions transferred to fresh medium and kept under room conditions made normal growth.

It may thus be said quite definitely that temperature is not a closely limiting factor for this fungus. Even at the very unfavorable temperature of 37° C., when development is greatly inhibited, it requires prolonged exposure to kill the culture. Likewise, although it is clear that the conidia

cannot develop much beyond germination at a low temperature, they can nevertheless survive periods of unfavorable temperature for a considerable time and be ready for rapid growth when again put under favorable conditions,—a characteristic that, under field conditions, probably is important for the survival of the organism.

Moisture

Very early in the experimental work, it was noted that an abundant supply of moisture is necessary for typical growth of *C. purpurea*. In its natural habitat the organism has ready access to the plant juices, and the sphacelial stage develops its myriads of conidia in a constant drip of "honey dew." Liquid media, however, did not prove to be favorable for growth. Sturdy mycelium was obtained only upon solid or semi-solid substrata. Ordinary percentages of agar, even to three percent, dried out too rapidly to permit normal vegetative development, but when the percentage was increased to six percent or more, thus insuring a moisture supply slowly set free throughout a long period of months, even of years, a much heavier growth was obtained than on any liquid medium. The factor of hardness might be thought to enter here, but it is of negligible significance compared to moisture supply. Hyphae of *C. purpurea* never penetrate the agar to any appreciable extent. Once the growth is mature, the entire mycelial mat can be stripped intact from the surface without great difficulty. After these facts had been ascertained, the general method was to supply adequate, constantly available moisture to the fungus by means of a medium with an agar content of at least six percent.

Pressure

In an attempt to induce the organism to grow against pressure within the agar mass, solid materials were added at the time of inoculation. In one experiment, bits of rye straw, soaked in spore suspension, were stirred into the nutrient agar just before the gel set. Those which chanced to be upon the surface developed normal mycelium and conidia, but no growth took place within the mass. In another case, bits of glass tubing about 3 by 8 mm. were similarly used, care being taken to have the lumen filled as far as possible with spore suspension as each tube was dropped into the warm agar. Evidently the air in the aqueous suspension within the tubing was sufficient to support initial growth in and around the tubing, for very soon the agar was split in all directions so that pressure was no longer a factor. The resulting growth, although of normal type, differed in no way from that of an ordinary agar surface culture.

Sterilized kernels of rye were used for a third effort to put the growing fungus under pressure, half of them being first dipped into melted hard paraffin. A teasing needle was forced about two-thirds of the way down from the tip, to make an opening. Into this canal a small amount of spore

suspension was injected by means of a fine Luer syringe with Yale needle. Each kernel, as it was inoculated, was pressed, base down, into a hard agar gel that contained no nutrients, being used merely to hold the grains upright. All kernels developed heavy cushions of mycelium at the mouth of the canals which, in the non-coated ones, proceeded no farther. The paraffined ones, however, proceeded in approximately a month to round up this mycelial weft into bundles of close-packed hyphae very rich in oily material. These cylindrical structures measured about 0.5 by 2.5 mm. and, on exposure to light, readily assumed the characteristic carrot red (R) [2] color, but they made no farther progress toward sclerotial formation.

Light

No indication of heliotropism has ever been seen in this fungus. Hyphae show no tendency to curve in any particular direction, even when the mycelium is at its greatest height.

The influence of light upon color production has been rather fully discussed by the writer in a previous paper (23). As there stated, its effect upon the mycelium of *C. purpurea* was to change the growing mat from whitish to a beautiful "carrot red." It should be emphasized here that throughout the work, whenever it seemed advisable, cultures were grown in triple series: in the dark, in diffused light, and in direct sunlight. When grown entirely in the dark, no aërial mycelium formed on any of the many nutrients used, nor did even a faint trace of the characteristic red color appear. The culture surface remained smooth, of greasy or waxy appearance, although conidia were abundant on the mycelial web. When cultures received a moderate amount of diffused light; *e.g.*, when kept in a cupboard with glass doors but at some distance from the source of light, they were able to develop characteristic aërial hyphae, "tilleul buff" in color, but they never formed the red color. When grown upon a table in diffused light somewhat stronger than that which reached the cupboard, aërial mycelium formed and became faintly pink, but the coloration proceeded no farther. When cultures were kept continuously for six weeks under a 100-watt daylight bulb at a distance of from twelve to fifteen inches, only a faint pink color was developed. Temperature was checked and found to be well within the optimum range at all times.

Earlier work of the writer (23) established the fact that color production in this organism is directly dependent upon the presence of the shorter rays of the spectrum. To extend this experiment a study was subsequently made to determine the effect of ultra violet rays upon cultures exposed to full radiation from a Cooper-Hewitt mercury-vapor lamp. Five series of cultures were run with varying conditions of exposure.

Series I tested the effect upon cultures about two weeks old growing on standard agar in small capsules. These were exposed directly at a distance

[2] Ridgway, Robert. Color standards and color nomenclature. XIV: 7′ : b.

of 50 cm. from the lamp, using 220 volts, 5.3 amperes, for 15 seconds, 30 seconds, and one minute, respectively. They were then returned to room conditions and, when no effect was found after a three-day interval, all were again exposed as before. Under these conditions growth was checked in direct ratio to the time of exposure. A third, similar exposure seemed to have checked permanently any extension of the mycelium, but the cultures were not killed. In from two to four weeks after they were returned to room conditions, a thin mat had covered the entire available surface. The cultures exposed 30 seconds and 60 seconds developed a slight pinkish color after the first exposure, but this never became intensified and it disappeared after the second exposure. In Series II, the above time intervals remained the same and a two-minute exposure was added. As before, the growth of the vegetative mat was much hindered as compared with the controls, but in no case was the culture killed. Within three weeks after they were replaced under room conditions, sparse growth covered the entire surface of the medium. The slight color induced by the first exposure of the 30 second, one minute, and two minute cultures soon faded.

In Series III, the effect of a single long exposure was tested instead of three short ones as in the two preceding series. Thrifty cultures two weeks old, growing on cornmeal agar in Petri dishes, were exposed at 21 cm. distance to radiation at 4.3 amperes, 150 volts, for 5, 10, and 15 minutes, respectively. Surprisingly, none were killed, but the 15 minute culture was able to make very little subsequent growth. Feeble as it appeared, however, good transplants could be made from all parts of its surface. The temperature of the air over the cultures exposed so long reached as high as 58 degrees C., thus again showing that this fungus is able to survive a very high temperature for an exposure of at least several minutes. In Series IV, the same type of cultures were used as for Series III. The 10 and 15 minute time intervals were repeated, and exposures for 20 and 25 minutes were also included. The lethal period lies between 15 and 20 minutes. Death may be due in large measure, however, to abnormally high temperature, as the heat could not be overcome readily with the apparatus at hand. Series V was planned to test the effect of the rays upon germination of the conidia planted upon standard medium. The lamp was operated at 650 watts, 5 amperes, 130 volts, at a distance of 25.4 cm. Direct exposures were made for 30 seconds, one minute, and two minutes. All conidia were killed by an exposure of one minute, but practically all withstood 30 seconds. The growth of the vegetative mat was slowed down for several weeks, but otherwise was normal and eventually equalled that of the controls.

The results obtained from ultra violet radiation, applied by a mercury-vapor lamp, may therefore be summarized as follows:

a. Actively growing cultures one to two weeks old survive three exposures of two minutes each at a distance of 50 cm.

b. Such cultures survive a single long exposure for from 15 to 20 minutes at a distance of 21 cm.

c. Conidia are able to germinate, with subsequent growth, after 30 seconds exposure at a distance of 25.4 cm.

d. Color induced by these exposures was faint and temporary; it never approximated that produced by sunlight, and it never withstood the second exposure.

e. Radiation had no effect upon the development of sclerotia.

Conidia of *Claviceps purpurea* are thus somewhat more resistant than those of *Glomerella cingulata* (Stoneman) Spaulding and von Schrenk for which the dose is 15 seconds, as reported by Stevens (36, 37). This time interval he also found fatal to the aërial mycelium, although the colonies survived a much longer exposure; *viz.*, 4 to 15 minutes. However, the striking stimulus to formation of fruit bodies which he repeatedly obtained has never occurred in the writer's cultures.

The remarkable color produced in the hyphal walls upon exposure to direct sunlight; *viz.*, carrot red, naturally lead to an investigation of the nature of the pigment. Numerous solvents were tried in an attempt to isolate the coloring matter from fresh or dried mycelium (table 2). The deeply colored fungus mat was stripped from the surface of the hard agar medium and carefully freed from any clinging fragments of the nutrient. Part of it was used at once in the fresh, moist condition. Part was quickly dried in a desiccator over calcium chlorid, a method which allows the full color to be retained in the mycelium. In this dried form, such a colored mycelial mat can be kept indefinitely.

Small amounts of the material, 15 mg. per cc. of liquid, were placed in the selected solvents at room temperature, records being taken at intervals of from 20 minutes to 24 hours. In the case of dried mycelium it was found impossible to extract the pigment satisfactorily with any of the solvents tested. At the end of 24 hours practically all fragments retained their full color. They were then heated on a water bath for 20 minutes at 60° C., but the color was not affected. The tubes containing alcohol and acetone were then brought to the boiling point, but with very slight extraction of the color of the fungus in either solvent.

The pigment from fresh, moist mycelium was likewise extracted with difficulty by most of the common solvents. As reported previously (23) absolute alcohol and acetone were the two most effective reagents in dissolving the pigment although, as table 2 shows, several others also removed it to a greater or lesser degree. It is noteworthy, too, that when the thoroughly dried mycelium was soaked for a short time in water and then tested with the various solvents, it behaved in every way like the fresh mycelium, hence it does not appear that the drying process changed the nature of the pigment. Therefore, the difference in extraction of the pigment from moist versus dry mycelium resolves itself into a question of penetration of the solvents which apparently is much less in the case of the dried material.

TABLE 2

Reagent	Effect: Fresh Mycelium	Effect: Dried Mycelium
1. Acetone	Color extracted in 1 hour	No effect in 24 hours
2. Alcohol, ethyl, absolute	Color extracted in 1 hour	No effect in 24 hours
3. Alcohol, ethyl, 75%	No effect in 24 hours	No effect in 24 hours
4. Alcohol, ethyl, 50%	Effect very slight in 24 hours	Very slight effect in 24 hours
5. Alcohol, amyl	No effect in 24 hours	No effect in 24 hours
6. Alcohol, methyl	Color extracted in 1 hour	No effect in 24 hours
7. Benzol	No effect in 24 hours	No effect in 24 hours
8. Carbon disulphid	Color quickly changed to rose-pink permanently with no extraction	No effect in 24 hours
9. Carbon tetrachlorid	No effect in 24 hours	No effect in 24 hours
10. Chloroform	No effect in 24 hours	No effect in 24 hours
11. Ether	Effect very slight in 24 hours	Very slight effect in 24 hours
12. Ethyl acetate	Color extracted in 1 hour. Mycelium shrunken, brown	No effect in 24 hours
13. Ethylene chlorid	Color extracted in 1 hour	Very slight effect in 24 hours
14. Formaldehyde, 40%	No effect in 24 hours	No effect in 24 hours
15. Furfural	No effect in 24 hours	No effect in 24 hours
16. Glacial acetic acid	Color gone in 1 hour bleached. Liquid still water white	Only partly bleached in 24 hours. Completely in about 3 days
17. Petroleum ether	Very slight extraction in 24 hours	
18. Toluol	No effect in 24 hours	No effect in 24 hours
19. Xylol	No effect in 24 hours	No effect in 24 hours
20. Alcohol-ether-chloroform in equal volumes	Color extracted in 1 hour	About ½ extracted in 24 hours oily layer on surface.
21. Pyridine	Color extracted in 1 hour	No effect in 24 hours

The oily residue obtained by evaporation in those cases where the pigment was dissolved was very suggestive of carotin in solution, but it failed to give the characteristic test for carotin when treated with a solution of antimony trichlorid. It does, however, give a characteristic test for ergosterol when brought into contact with the antimony trichlorid: pink, changing to a permanent blue. In mycelium comparable in every way except that it had not been exposed to light and therefore was not colored, the ergosterol test was very faint; hence it seems quite evident that the content of this lipoid had been increased by exposure to light.

Transpiration

An extensive study was made of the effect of inducing an increased amount of transpiration by supplying a forced current of dried air to the fungus. Cultures two to three weeks old were used, growing upon standard medium in deep agar slants in liter Erlenmeyer flasks. The set-up was aspirated by means of a Chapman filter pump. Indrawn air was sterilized by passage through a 1–1000 solution of bichlorid of mercury, dried by passage through several tubes of sterile calcium chlorid, drawn to the bottom of the slant by a long intake tube, then out through a short tube in the neck of the flask. This arrangement worked satisfactorily with the cultures attached. The intake was constantly dry, the exit tube constantly moist,—at times even quite wet. With frequent changes of the calcium chlorid

tubes, thus keeping the incoming air thoroughly dried, the moisture in the medium evaporated much more rapidly than is usually the case in similar flask cultures with ordinary cotton plugs. The vigor of the cultures was not impaired by this rather drastic treatment. They continued to thrive and developed a thick mat of growth appreciably heavier than that of the controls.

When the set-up was so placed that the fungus was exposed to light, the reaction was characteristic; the carrot red color quickly appeared, then became obscured by a new overgrowth which in turn became deeply red. This succession occurred several times, the period between the appearance of colored mycelium varying more or less according to atmospheric conditions. The age of the cultures did not appear to be important.

After such constant aëration for three weeks, the medium had dried to approximately one-eighth the original volume, and the usual pseudosclerotia began to appear, embedded in the mycelial mat which had become deeply convoluted during the drying process. The experiment was allowed to run until no further change could be noted; *i.e.*, the spread of the mycelium had reached its maximum, and evaporation had apparently ceased. The flask was then broken and the adhering medium, now dense and leathery; was carefully whittled off as closely as possible, leaving the fungus mat intact. As the photograph shows (Pl. VII, fig. *c*), the surface was thickly dotted with the embedded pseudosclerotia which were approximately twice as large as those normally found in aged cultures. Many of them were as large as 3 mm. in diameter. Numerous ones were removed for histological study, but they differ from those previously described only in the larger size. It is concluded, therefore, that there are some significant changes produced in the vegetative condition of the organism as a result of the increased transpiration; but that either the reaction could not be carried far enough, or the combination of other stimuli, internal or external, was unfavorable for the production of sclerotia.

Oxygenation

In studying the relations of oxygen to *C. purpurea*, cultures were placed under a variety of conditions with respect to this element.

Hydrogen Peroxid. This substance was found to be inimical to the growing fungus under any arrangement for its use that was attempted. If placed in direct contact with the mycelium, the threads were quickly blackened. By means of a Glaseptic Nebulizer,[3] an extremely fine spray was added to the atmosphere above cultures growing in glass capsules, but in this case, too, the mycelium was soon darkened and further growth was inhibited. Increasing the amount of the hydrogen peroxid spray

[3] "Glaseptic Nebulizer" is the trade name of an atomizer, kindly provided by Parke, Davis & Co., Detroit, Mich., which gives an exceedingly fine spray and requires only a very small amount of the experimental material.

correspondingly increased the amount of injury, and at no point could any favorable stimulation be observed.

Enclosed Oxygen. Cultures in capsules and test-tube slants were placed in Novy jars which were then evacuated by means of an air pump and refilled with varying proportions of air and of oxygen from commercial tanks. The oxygen was added in 30 percent, 50 percent, and 80 percent amounts; the remaining percentage was air. An atmosphere of commercially pure oxygen was also used, evacuating the jar and allowing the tank pressure to fill it. It soon became evident that an enclosed atmosphere of high oxygen content does not favor the vegetative processes in this fungus. The mycelial mats in the jar containing approximately 44 percent oxygen and 56 percent nitrogen did not fall much below the controls, but when exposed to light they showed decidedly less coloration as compared with the controls. In the atmosphere of 60 percent oxygen, 40 percent nitrogen, there was a decrease in rate of growth and the color was also much paler than that of the controls. In 84 percent oxygen, 16 percent nitrogen, harmful effects were very marked; further growth was inhibited. With 100 percent oxygen, the conidia lost their power to germinate. Cultures that were first grown for a week under room conditions before placing in the jar of pure oxygen, were soon inhibited and were dead in approximately two weeks from the time of exposure.

Flowing Oxygen. Simultaneously with the foregoing tests, experiments were in progress on the effect of a continuous stream of oxygen at atmospheric pressure over the surface of cultures. For this a slightly more complicated apparatus was necessary than that used in the transpiration work, as shown in the photograph (Pl. VIII, fig. *c*), but it was similarly arranged.

A. Moist Oxygen Current. Gas from the tank was forced by its own pressure through a deep tube of 10 percent sulfuric acid, then through several tubes of sterile, distilled water, and finally over the surface of a thrifty week-old culture growing on deep agar slant in a liter Erlenmeyer flask. Gas intake was regulated by means of bubble count, about 100 per minute, so that no undue collection of moisture occurred as compared with the controls, but the nutrient material naturally did not dry as rapidly as that in the controls. The entire set-up was placed in the same southern exposure at a fourth-floor window facing on the open campus, and allowed to run continuously for four weeks.

A decided acceleration of both mycelium development and color formation occurred in the cultures thus treated. A heavier mycelial mat formed, and the characteristic carrot red color was produced from 24 to 48 hours earlier in the treated cultures than in the controls. Thus it regularly happened that treated cultures became fully colored and the color was obscured by the new overgrowth before the controls had developed their full coloration. The repeated cycle of tilleul buff-carrot red-tilleul buff was

always more marked in the treated cultures than in the controls. In the culture treated with oxygen the red color appeared three times with intervals between when newly formed mycelium obscured it before it, in turn, became colored. In the controls this cycle occurred only twice in the same period of time. Due to abundant moisture, the mycelium spread rapidly down the sides of the medium in the treated culture as the agar receded from the container. In this mycelium and in that on the thinner part of the slanted agar in the flask, pseudosclerotia were very numerous, deep red in color, and varied in size from 1.0 to 3.1 mm. in diameter. Not only did they appear nearly a week earlier, but they were also considerably larger than were those in the controls.

Despite this remarkable stimulation in the early stages of the treated cultures, it later became evident that through long exposure to the moist current of oxygen the fungus had lost its ability to develop the characteristic color. At the end of the fourth week it had seemingly run its course; the whole surface was now grayish while the control was still in its prime. The flasks were therefore detached and returned to the cupboard. Two weeks later, however, a most remarkable resumption of growth had occurred in the culture which had been treated: whereas the surface had been of a pasty, gray appearance when the culture was removed from the window, it now showed very marked, vigorous, upright hyphal development over the whole surface, with heavy mats extending down the sides. When again placed in direct light, the deep, rich carrot red color quickly appeared over the entire surface. This color continued for several weeks thereafter, and the mat became deeply plicate by the rapid development of hyphae as is shown in the photograph (Pl. VIII, figs. *a*, *b*).

B. Dry Oxygen Current. To test the effect of a stream of dry oxygen, the preceding apparatus was varied only by passing the stream of sterile, washed gas through a series of tubes of sterile calcium chloride before it entered the culture flasks. All other conditions duplicated those used with the moist gas. Results were somewhat different when the dry current was used. Instead of the treated culture leading, it now lagged about twenty-four hours behind the development of the controls. Succession of color and overgrowth was less marked from day to day under the dry gas than it had been under the moist current, and the controls passed through the color-overgrowth-color cycle about twenty-four hours earlier than did the treated culture. It is believed that the delay may be attributed to the effect of drying, rather than to that of the oxygen *per se*, for at the end of the experiment the treated culture was more vigorous than the controls. No harmful effect was apparent here, such as had occurred under the prolonged moist current; indeed, the culture had spread considerably wherever the medium contracted from the glass, and this mycelium, as well as that on the thinner portion of the slanted surface, was thickly dotted with pseudosclerotia showing characteristic color among the thrifty upright hyphal clumps. On

the whole, the dry oxygen seemed more favorable for increased mycelial development than did the moist oxygen. The apparatus was allowed to run several weeks, until the agar had practically disappeared. The mycelial mat had shrunk to about one-fifth the original area, and was correspondingly thickened and cortex-like. In cross-section, it showed a structure quite similar to the pseudoparenchyma of the natural sclerotia. In this mycelium the bodies were firmly embedded. Both of the foregoing series, with moist and with dry oxygen, were checked and substantiated the findings as given. They were also repeated, varying (*a*) the amount of food available to the fungus, and (*b*) using tissue transplants instead of spore suspension; but results in each case corresponded closely to the detailed account given above.

C. Oxygen Plus Carbon Dioxid. Since it had been found (*a*) that streaming pure oxygen stimulated vegetative development, and (*b*) that enclosed oxygen gave an unfavorable environment, a test was planned to study the effect produced by a stream of 50 percent oxygen. The same set-up was used, except that it was attached to a tank of carbon dioxid as well as to the tank of oxygen. The gases were mixed in closely approximate amounts (volumes), adjusted by means of bubble count, about 100 per minute. Development under this mixture was not different from that of the control during the first week. Then the treated culture lost its color, not from the usual formation of new overgrowth, but by fading or a real bleaching. The mat spread no farther, the surface appeared abnormal, of a grayish hue which remained the same throughout and presented a striking contrast to the control which was passing through the repeated color-overgrowth-color succession. No permanent injury had been done, however, for again after a two weeks rest from the current, the treated culture had resumed growth and continued to thrive for several weeks thereafter, giving characteristic color reactions upon exposure to light.

Throughout the experimentation with oxygen, the same facts stood out and may be summed up as follows:

1. In cultures grown in streaming oxygen, the growth of the mycelium is decidedly accelerated and color formation is promoted.

2. The characteristic pseudosclerotia are formed much sooner than under any other conditions, and are appreciably larger.

3. Further differentiation of tissue is lacking; and, somewhat later, control cultures reach a stage of development approximately equal to that of the treated cultures.

4. Oxygen gas, when available, is used by this fungus in large amounts for more rapid metabolism as shown by the marked increase in mycelium under such conditions, where a web two to three millimeters thick is formed, as compared to the average of one millimeter.

5. The accumulation or presence of carbon dioxid has a somewhat inhibitory effect upon the fungus, as shown in the experiment with flowing

gas, 50 percent oxygen and 50 percent carbon dioxid, and as also shown, presumably, in the experiment with enclosed oxygen.

Homothallism

Much successful work has been reported recently on the production of reproductive organs through the crossing of mycelium derived from single spores or from mycelial strains in such fungi as the rusts, several species of *Phytophthora* (2, 3, 4, 5), and *Monilia sitophila* (Mont.) Sacc. (9). Since *Claviceps purpurea* had proven to be unresponsive to the stimuli provided for inducing the formation of sclerotia, it seemed possible that in the case of this organism also heterothallism might figure in an important way in producing the stromata on which the perithecia are borne.

However, rather strong indications of homothallism have resulted from the present study, in attempting to isolate plus and minus strains. For preliminary work, the sclerotia used had been obtained from ten sources of commercial "Ergot." Three were from Spain, two from Germany, one from Portugal, one from Switzerland, and three from the United States. Bits of tissue were isolated aseptically from each of these, and from each of the resultant cultures a single-spore culture later was isolated. These ten cultures were then repeatedly crossed in the forty-five combinations. No antagonism was found. In every case, the hyphae from each source intermingled as they spread over the surface, forming a mat of normal appearance that could in no way be distinguished from that of either of the originals. These ten single-spore mycelial cultures have been maintained for more than a year, with paired cultures made at intervals of about three months, but no indication of sexual grouping has ever occurred.

From germinating sclerotia, mature asci were obtained for a similar study of single-ascospore cultures. These fine, thread-like, hyaline spores, which measure about 1.0×14 μ, are exceedingly difficult to separate from each other. A single ascus can be drawn up by means of an extremely fine capillary pipette, but when the slender spores are freed from the ascus and sprayed over the nutrient surface, it has proved impossible by any means yet devised to prevent their clumping in groups of two, three, or more spores. They are, moreover, so fragile that, even when a single ascus has been isolated in a little water, any attempt to separate the spores resulted invariably in broken or disintegrated spores. In no case, working with seventy-nine individual asci, was a successful isolation of a decisive number of single spores attained from a single ascus. The technical difficulties here can scarcely be over-emphasized though it is fully realized that, unless they be surmounted, no satisfactory conclusion can be drawn as to the possible heterogeneity of the ascospores.

In the course of this work a large number of cultures accumulated, for the ascospores grow readily when sprayed upon the agar surface. Some were from groups of ascospores, others were from single spores, but all

appeared identical both macroscopically and microscopically. Several of these cultures were used as the basis for further crossing experiments. Cultures I, II, III, IV were from the same ascus and consisted, respectively, of one, one, two, four ascospores. Cultures V, VI, VII, VIII, and IX were from another single ascus and consisted, respectively, of one, one, one, two, three ascospores. Thus seven cultures known to be from single ascospores *from three different asci*, and five cultures comprising two or more ascospores from the same ascus were maintained for a sufficient length of time to test and repeat the sixty-six possible combinations. Here, as with the conidial cultures, it was clearly seen that no combination resulted in any evidence of inhibition or acceleration. Cultures grew normally, whether both were inoculated in the same area of the medium or planted at opposite sides and allowed to meet. In no case could any tendency toward sexual grouping be discerned. The mat formed by hyphae meeting from separate inoculations differed in no way from that developed where crossing was made in the same area; nor could either of these be seen to differ from an ordinary saprophytic culture of a single ascospore or a single conidium.

Field Culture of Ergot

During the summer months of 1923 and 1924, a study was made of the practicability of growing ergot in field rye. In the fall of 1922, preparations were made by seeding three one-fourth acre plats with Rosen rye at intervals of two weeks, hoping thus to secure three blooming periods. All plats grew well, but in the spring not much difference was to be noted in their general appearance. The field planted earliest came into bloom first but the other two were very little behind it, so that all three had to be inoculated together during most of the flowering period, June 6 to June 14. To prepare the inoculum, spore suspensions from mature cultures of *C. purpurea* were grown in mass upon trays of agar medium for from four to five days. This gelatinous, conidial growth was then washed from the surface, filtered through sterile gauze, and made into a heavy suspension with sterile, distilled water. In the field this suspension was added to a tank of water and, by means of a pressure pump, sprayed in a fine mist over the blooming heads of rye. The whole spraying equipment was mounted upon a horse-drawn wagon. Sterile plates of standard medium were exposed each time to the mist at the beginning, midway, and at the end of the field spraying. They developed heavy growth over the entire plate, with surprisingly few contaminants showing. Spraying was repeated every second day until five inoculations had been given. The weather was troublesome, showers occurred on two of the five days during or soon after the spraying. However, a considerable infection was evident as the "honey dew" began to appear June 21, from which normal conidial cultures were obtained. Immature sclerotia were forming abundantly by June 29 and were beginning to show color when a definitely unfavorable period of weather occurred.

An extremely hot, dry interval was followed by a violent hailstorm July 5, which badly flattened the rye. It revived fairly well within the next few days, but during July 6–11 there were three disastrous wind and rain storms which left the rye badly lodged. Many of the developing sclerotia had been killed. Subsequent development was much less than had been indicated in June, but a fair amount of infection still persisted. The sclerotia, however, were rather stunted and mis-shapen, and the final yield was scarcely worth the trouble of harvesting by hand. While material results for the season were disappointing, yet it had been shown that mass inoculation could produce heavy infection.

The following summer considerable improvement was made in technique, but matters in general were handled as before except that a new, better sprayer was available, steam was added to make the water tepid, and glucose was added to the spray mixture with the idea that it might be useful as an adhesive agent. Four one-quarter acre plats were used: *a*, Rosen rye; *b*, Old Type rye; *c*, Spring rye; and *d*, Burt oats, an open-glume type of oats, said to be susceptible to *Claviceps purpurea*. Plats *a* and *b*, having been put in in the fall, came into bloom first, and were sprayed twice daily from June 17 to June 20, inclusive, a total of eight times. Plats *c* and *d* came into flower a few days later, and were sprayed twice daily June 24 to June 30, inclusive, except on June 28 when a heavy thunderstorm prevented. Throughout much of the time when the fungus should have been developing, the weather continued hot and dry with the result that very little infection was obtained in any of the plats. It was therefore concluded that such method of ergot production is not likely to be of practical use in America, where the cost of hand labor is prohibitive as compared with, for example, the conditions under which Hecke carried on his experiments. As stated elsewhere (p. 52), large, well-formed ergots can be produced by means of hand inoculation, even when conditions are not especially favorable, but this could not prove practicable on a large scale.

Pharmaceutical Characteristics of Mycelial Extracts

The active principles of ergot are chiefly the specific alkaloids (ergotoxine and ergotamine), histamine, and tyramine. The relative amount of each present in an extract of ergot is judged by physiological methods, of which the cock's comb test is official in the United States Pharmacopoeia. In general it may be said that the specific alkaloids are well shown by the cock's comb test; the oxytocic test, using isolated guinea-pig uterus muscle, indicates more closely the histamine content; while the blood-pressure test will indicate the presence of all three but, because of the antagonistic action of histamine, is not a reliable quantitative index of activity.

Rather numerous tests were made of extracts prepared from *C. purpurea* grown upon various media, in order to compare such saprophytic growth with the natural parasitic type. Some of the samples were subjected to

all three of the tests but, since the cock's comb test is official and is considered by many pharmacologists to be the most reliable for indicating the actual value of an ergot extract, many were tested only by that method.[4] The many details of the preparation of each sample need not here be given. Suffice it to say that each represents a Fluid Extract made from saprophytic growth of a culture of *C. purpurea*, approximating as closely as possible the strength of the official U.S.P. preparation. Where results are indicated by figures, it is to be understood that 100 percent is typified by U.S.P. Fluid Extract.

TABLE 3. *Physiological Tests of Saprophytic Mycelium of Claviceps purpurea*

Sample Number	Culture Number	Cock's Comb Test	Oxytocic Test Test	Blood Pressure Test
1	168	30–40 percent of Standard	400 percent of Standard	Not characteristic
2	169	50 percent of Standard	40 percent of Standard	
3	171	About 60 percent of Standard	40–50 percent of Standard	Rather characteristic
4	169	About 50 percent of Standard	100 percent of Standard	Marked depressor
5	169	50–40 percent of Standard	Fair	Equal to Standard
6	168	About 40 percent of Standard	Fair	Nearly Standard
7	177	About 75 percent of Standard	None	Not characteristic
8	177	Typical. 60–75 percent of Standard	Marked activity	Marked depressor
				Moderate depressor
9	177	Fair. 25–40 percent of Standard	Fair	Not characteristic
10	177	75 percent of Standard		
11	181	40 percent of Standard		
12	169	50 percent of Standard		
13	181	65 percent of Standard		
14	181	50 percent of Standard		

The Standard referred to in the above table is the reaction produced by the official U.S.P.X. Extract of Ergot. A study of the above data will show that Culture 177, used throughout this work, gave the highest yield of the characteristic alkaloids. The percentage is found to vary according to the medium used; *e.g.*, the only difference in nos. 7, 8, 9, 10 was the medium upon which the fungus was grown.

A study of table 3 will show that the extracts varied considerably in their content of the active principles, but several showed a typical reaction approximately equal to three-fourths that of a standard ergot extract; *e.g.*, Nos. 7, 8, 10, and 13. The studies were carried far enough to show considerable encouragement in this method of securing the active principles from the mycelium. It could be employed successfully in case it should become necessary to depend upon artificial culture of the organism for

[4] For physiological testing of the samples listed in table 3, the author is indebted to L. W. Rowe of Parke, Davis and Company.

medicinal purposes. The significant fact is that this is the first record of a successful attempt at securing the extract from any part of the fungus other than from the sclerotium. Because of practical considerations, nothing more was done on this phase of the work.

General Discussion

In the foregoing studies, *Claviceps purpurea* under saprophytic maintenance has been found responsive in marked degree to several factors of environment, such as control of food or temperature, and especially so with regard to oxygen, moisture, and light. Control of vegetative development by high optima of these factors has met with unprecedented success, a heavy, cortex-like layer having been secured at will. In section, this layer is strongly suggestive of the structure of a cross-section of the natural sclerotium, but it has not yet been induced to round up into definite sclerotia.

The effects of light upon fungi vary greatly in different species, from a factor of great importance to one of no moment. Generally speaking, prolonged exposure to strong light has been regarded as detrimental for successful vegetative development. Early mycologists believed light to be of much importance in the development of morphological structures in fungi, and its absence was held accountable for the formation of great masses of sterile mycelium in cellars and caves. Brefeld (7) was the first one to study the question scientifically, and found that while some species can accomplish feeble fructification in darkness, many others remain sterile unless given at least a brief exposure to light. Even so slight a duration as two hours he found sufficient to incite the formation of reproductive bodies which then made normal progress in the dark. Klebs (18, 19) found light to be of the utmost importance in the development of fungi and he stressed the need for investigating the effect of this factor upon each organism studied. Coons (8) reported that light is a strong determining factor for pycnidium production in *Plenodomus fuscomaculans* (Sacc.) Coons and, like Brefeld, he found that a short exposure is sufficient to bring about the desired result. Robinson (28), working with *Pyronema confluens* (Pers.) Tulasne, states that no reproductive structures occur except in the presence of some illumination, although the minimum of light energy required is small. Angell (1), in a study of pigment production in *Macrosporium porri* Ell., considers light to be wholly without influence. In the writer's work on the ergot fungus, the best development was obtained while the organism was exposed to the full influence of direct sunlight. Bonns (6) does not state the conditions under which he grew *C. purpurea*, except that his cultures were left at room temperature; but he mentions the occasional appearance of color, so it is probable that they were not grown in the dark. Kirchhoff (17) grew this organism entirely in darkness, and held light to be unnecessary for typical development both of mycelial growth and of his

so-called "artificial sclerotia." His result is decidedly at variance with the work of the writer who has found that light exerts a profound influence in such cultures, favorably affecting the growth and producing a most striking color development in the mycelial walls. No other stimulus caused these effects, whereas they were invariably induced by exposure to sunlight even for so brief a period as one hour. It does not appear that such color is due primarily to ultra-violet rays; indeed, it seems evident that the blue rays are the more effective for chromogenesis. Color was not produced by exposure to the ultra-violet rays from a Cooper-Hewitt mercury-vapor lamp, with subsequent removal to darkness, but a culture exposed to direct sunlight no more than one hour would later develop the characteristic color even when removed to the dark chamber. It was of considerable interest to note that no purple color, as reported by Bonns (6) and by Kirchhoff (17), has ever occurred in the cultures of the writer. Shades from "coral pink" to the characteristic, brilliant "carrot red" were readily obtainable according to the intensity of the light given, but no trace of purple ever was seen.

The lipoid material containing the pigment appears to be very rich in ergosterol. When it was extracted from the colored mycelium, as an oily, amber-colored liquid, and tested with antimony trichlorid, the characteristic evanescent pink, followed by a permanent blue color was obtained (10). Mycelium in every way comparable to this, except that it had not been exposed to light and hence was not colored, was similarly extracted and tested. It gave a faint reaction for ergosterol, but it was very slight as compared to that obtained from the colored mat.

Among the essential conditions for all forms of life, oxygen must be regarded as outstanding. There is, of course, a wide variation in the optimum requirement for different organisms, even for different stages of development of the same organism, but there are few, if any, species in existence that can be considered strictly anaerobic. In its relation to fungi, oxygen has been the subject of a great many studies. Among others, Angell (1) reported that oxygen is essential for pigmentation in *Macrosporium porri* Ell. Fellows (12) stated that *Ophiobolus graminis* Sacc. is not much affected until the oxygen content falls below 6 percent, when growth is greatly reduced. Leonian (20) studied the oxygen requirements of 20 species of Sphaeropsidales. Decreased oxygen supply suppressed fruiting in 3 of the species, reduced it in 11 species, and had no effect upon the other 6 species.

In the case of *C. purpurea* oxygen is a prime necessity. In any proportion, even to 100 percent, the fungus can utilize it, providing there is free circulation. Enclosed oxygen or enclosed air is unfavorable for good development of this organism, possibly because of carbon dioxid accumulation; but vigor of growth, thickness of mycelial mat, height of aërial mycelium, size of conidia, and size of pseudosclerotia were all more marked

in cultures given prolonged exposure to an atmosphere of flowing oxygen. While elaborate studies, such as those of Novy (26) and Novy and Soule (27) could not well be carried on with a fungus of this type, nevertheless it has been established that the organism thrives only with ample aëration. It is precisely this necessity for abundant aëration that made one phase of the study unattainable. Any attempt to grow the organism under mechanical pressure failed; cultures were unable to survive such conditions. This leaves unsettled the theory of Bonns (6) that cortex-like portions of the mycelial mat fail to round up into sclerotia only because they are developed without pressure; a plausible idea, but difficult to prove in this case.

So abundant has been the mycelial development obtained in these cultures, so constant have been the characteristics of such growth and its behavior over long periods of time, that the author feels certain *Claviceps purpurea* will yet be induced to carry through its full life cycle as a saprophyte. It is probable that such completion depends upon giving to the pseudosclerotia the environmental conditions comparable to those encountered by the natural sclerotium forming in the living host. It is not easy for one thoroughly familiar with this organism to believe that these pseudosclerotia are merely casual in the development of the mycelium. They are a constant, well-defined phenomenon the further study of which is fully warranted. However, nothing herein said is to be taken as a claim that they are, in truth, sclerotia, for such an opinion is not held. To make a claim that cannot be substantiated in every detail merely clouds the issue. Such an unwarranted assumption is made by Kirchhoff (17) in stating that he has obtained, in culture, artificial sclerotia of *C. purpurea*. The structures which Kirchhoff designates by this term are not new, nor are they at all uncommon. Hard, cortex-like, sclerotioid tissues are a fairly constant feature of the growth upon a variety of media, and just such portions as Kirchhoff describes can frequently be dissected, not only from the edges of the mycelium but in other parts of the surface. Several years before Kirchhoff's work was published, Bonns (6) recorded his opinion that the formation of such relatively hard crusts represents "a stage analogous to that found in the sclerotium," but he did not suggest that they be considered really sclerotia. It is recognized that size or shape alone are not features that determine the reproductive bodies developed in saprophytic culture. In the case of this fungus it is scarcely to be expected that they would conform closely in shape to the sclerotia formed in the rye flower. Nevertheless, they should be able to form the typical stroma, perithecia, and asci. Until there can be produced at will, by the growth of *C. purpurea* in culture, sclerotia that will later develop perithecia with viable ascospores, no one can maintain in fairness that he has succeeded in the production of "artificial sclerotia." To this extent, the writer takes definite issue with the claim so put forth recently by Kirchhoff.

No previous work has been done on the problem of heterothallic strains occurring in this species. As would be expected, matings between single-spore cultures from conidia of widely separated sources gave no significant results and merely made typical development. Even with the paired cultures from ascospores decisive conclusions cannot be drawn from the cultures so far obtained, yet the evidence points in the direction of homothallism. Although five single spores have never been secured from the same ascus, nevertheless all eight spores from an ascus have frequently been in a group of matings; *e.g.*, cultures from four, two, one, one ascospores, respectively, from a single ascus, were mated with each other and with similar cultures of one, one, two, three ascospores from another ascus. Neither in single-spore matings nor in the matings of the group cultures was there ever any indication of heterothallism. It may be possible eventually to isolate decisive numbers of single ascospores from a convincing number of single asci. Numerous crossings of such single-ascospore strains might then yield mycelium that will produce viable sclerotia. But these fragile, filiform spores are so easily destroyed in the manipulation necessary to isolate them that the difficulty in this case is much greater than with many types of ascospores or basidiospores such as those which were studied by Hanna (13), by Sass (29), or by Dodge (9).

The laboratory production of ergot of characteristic properties has been found to be quite feasible. To a somewhat less extent, this is true of field culture of *C. purpurea*. It is problematical, however, whether such laboratory production will be practical for medicinal application. The possibility of so utilizing cultivated ergot interlocks closely with conditions of European commerce and, being an economic question, need not here be discussed.

Summary

1. The studies of *Claviceps purpurea* herein reported have shown that under controlled experimental conditions, both physical and physiological, an unprecedented mycelial growth of this fungus can be obtained in saprophytic culture.

2. A nutrient synthetic agar was selected and used as a standard medium for securing a maximum mycelial development of this organism.

3. Of the stimuli tried, oxygen was found to have the greatest effect upon mycelial growth, also for increased size of the conidia and for earlier appearance and greater size of the pseudosclerotia.

4. The optima of heat, light, and moisture were determined, and reactions to other physical stimuli such as mechanical pressure and aëration are reported.

5. Sunlight produces a marked chromogenic effect upon the mycelium of this fungus by causing an intense coloration, Carrot red (R.). This greatly exceeded the faint trace of color produced by means of any other stimulant tried.

6. It has been shown in this and in a previous work by the writer (23) that the favorably stimulating rays for color production lie in the blue-violet region of the spectrum, and that the ultra-violet rays, although not markedly injurious to *C. purpurea*, have no special effect upon growth or reproduction.

7. Light has been found to increase the ergosterol content of the mycelium of this fungus growing in saprophytic culture.

8. No true sclerotia have been developed in culture, but the production of definite mycelial knots herein designated as pseudosclerotia is a constant phenomenon in properly nourished mycelium. It is suggested that these structures are possibly primordia of true sclerotia which have failed to reach their full development through lack of some essential conditions.

9. A partially sclerotioid mycelium is readily obtained on standard medium. Such tissue, however, does not develop the morphological characteristics of true sclerotia nor does it pass through a stage analogous to the stroma formation and subsequent germination of the natural sclerotia.

10. The experiments with paired cultures give strong indication of homothallism in *C. purpurea*.

11. *It has been demonstrated for the first time that this fungus develops in saprophytic culture the three chief active principles which are characteristic of the extracts made from the natural sclerotia; viz.*, ergotoxin, histamine, and tyramine; and that they are obtainable to an extent sufficiently large to be of economic significance.

12. Field demonstrations have shown it to be improbable that parasitic culture of *C. purpurea* on a large scale would be desirable, under prevailing conditions in Southern Michigan.

The studies on field culture of ergot, and the pharmacological work with extracts of saprophytic cultures, were carried out under the supervision of Parke, Davis & Co., Detroit, Michigan, to whom the author expresses her sincere appreciation for the privilege of using this material, and for their encouragement during the work on the problem herein discussed.

Grateful acknowledgment is made to Dr. C. H. Kauffman, under whose direction the work has been done, for his unfailing help throughout the years spent in these studies. Due to the illness of Dr. Kauffman, the final criticism of manuscript was kindly given by Prof. H. H. Bartlett and Dr. B. B. Kanouse, but the author assumes full responsibility for any errors that may appear.

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DESCRIPTION OF PLATES

PLATE VII

FIG. *a*. A section through a young pseudosclerotium, with the ordinary mycelial mat showing at each side. The lower, vacuolated area is overlain by denser layers, with loose weft of mycelium over all.

FIG. *b*. Detail of a center section of a typical pseudosclerotium. The vacuolated inner portion of pseudoparenchymatous appearance is overlain by denser layers of mycelial growth.

FIG. *c*. The dried mycelial mat from a culture which has undergone three weeks of forced transpiration. Pseudosclerotia may be seen embedded in all parts of the surface.

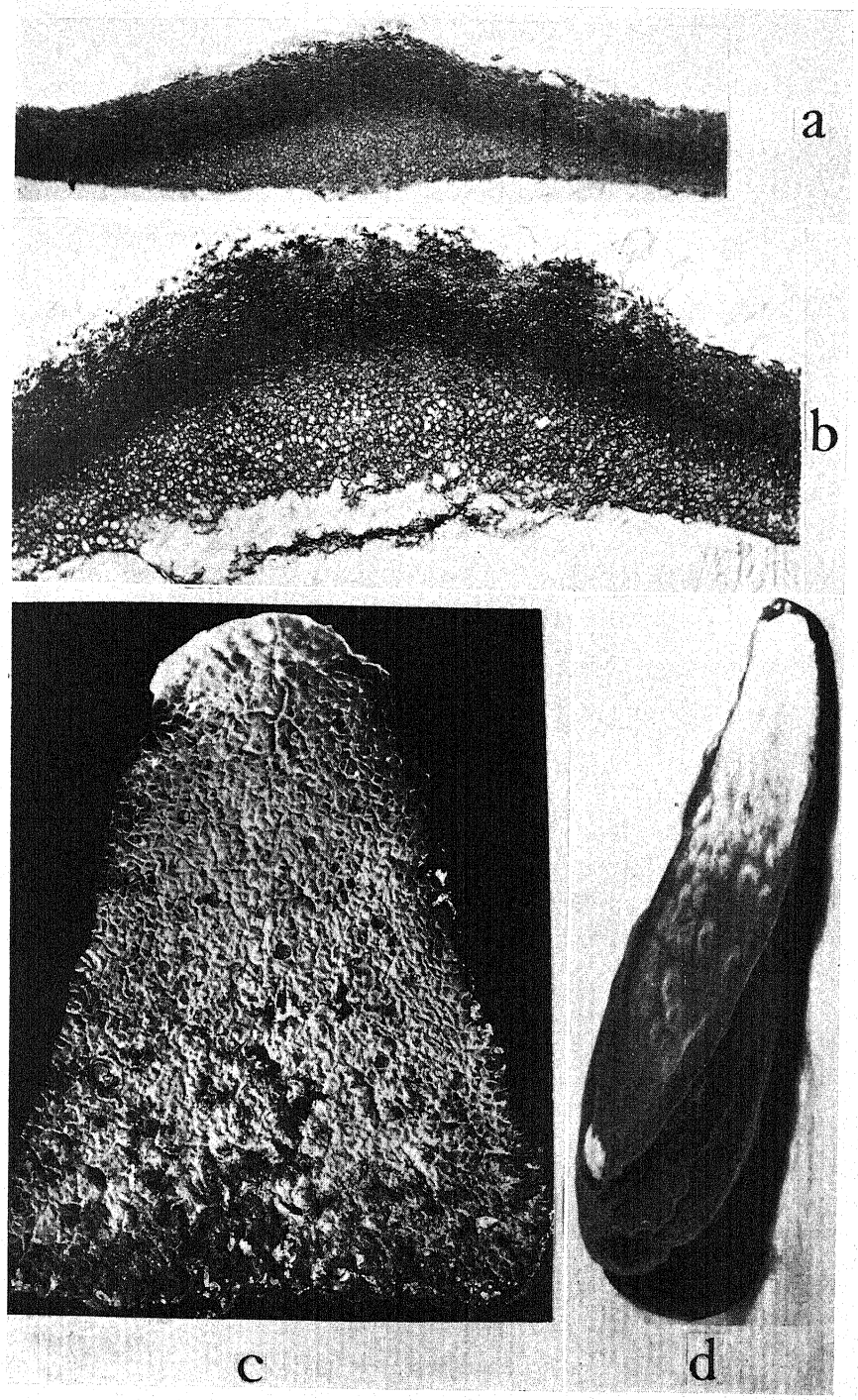
FIG. *d*. Pseudosclerotia forming in an actively growing culture having a surface about 10 × 3.5 cm. Typical mycelial overgrowth is seen starting over the thinner portion of the slant.

PLATE VIII

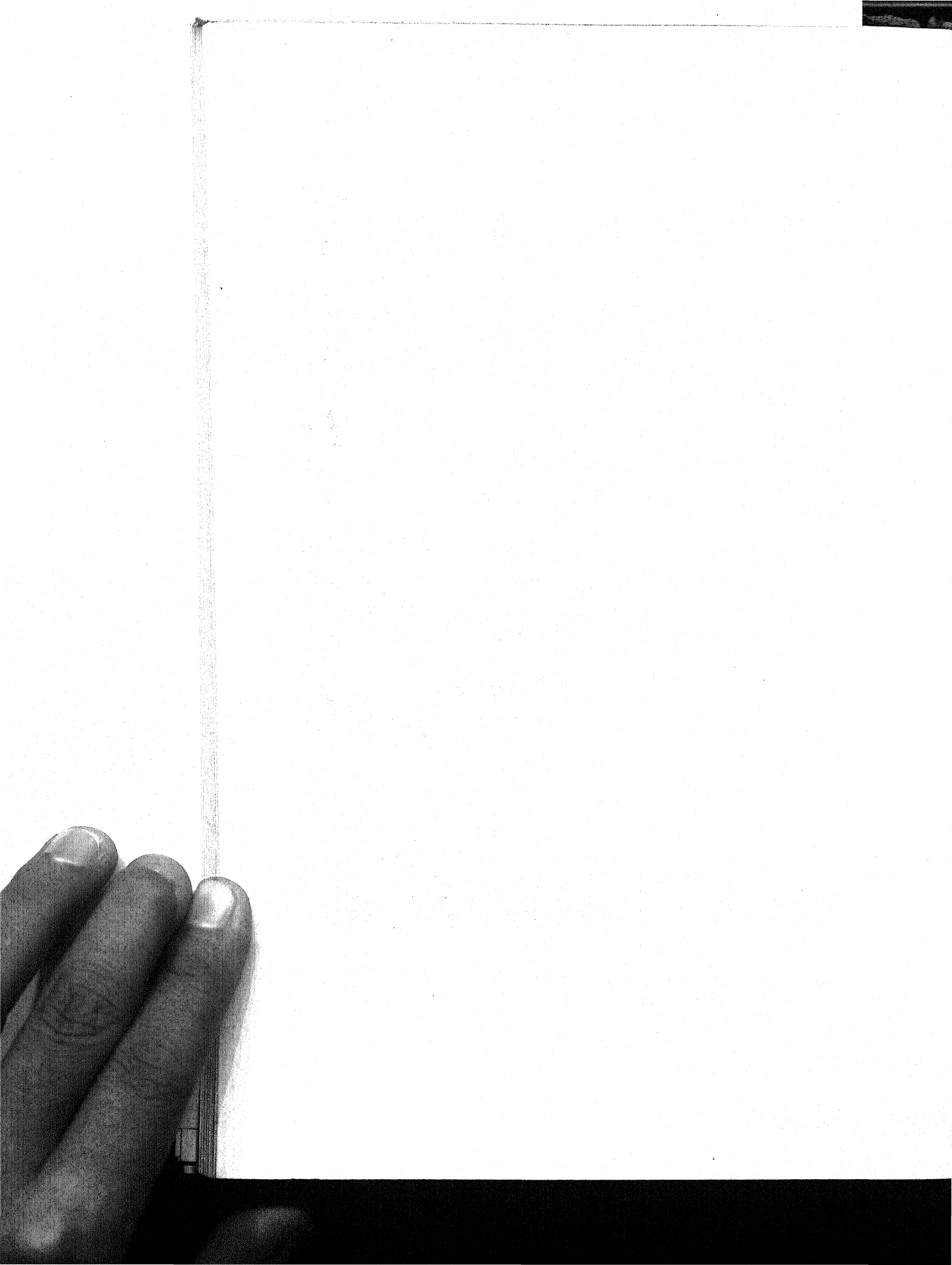
FIG. *a*. Culture exposed to continuous flow of oxygen about three weeks, showing heavy mat 2.5 to 3.0 mm. thick.

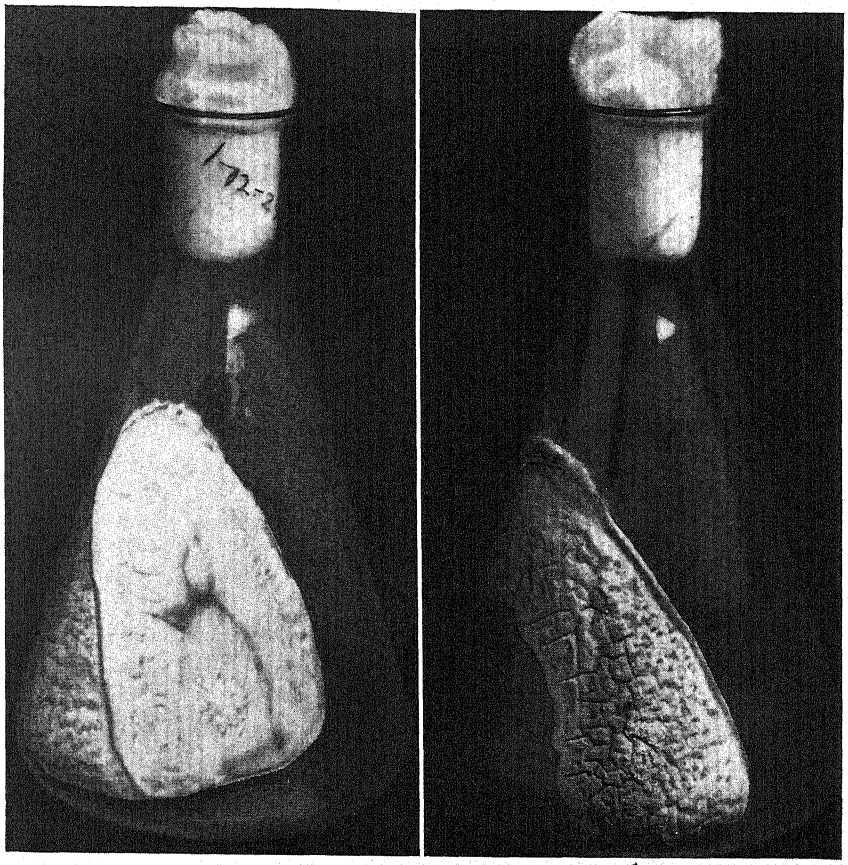
FIG. *b*. Rear view of culture shown in (*a*). The deeply plicate mat shows, at the right, the early stage of formation of pseudosclerotia.

FIG. *c*. The apparatus used for oxygen experiments showing, from the left, the supply tanks of oxygen and carbon dioxid, the washing tubes, and the calcium chlorid containers for drying the gas.

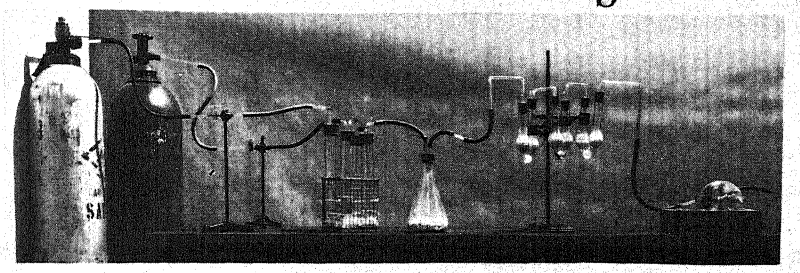


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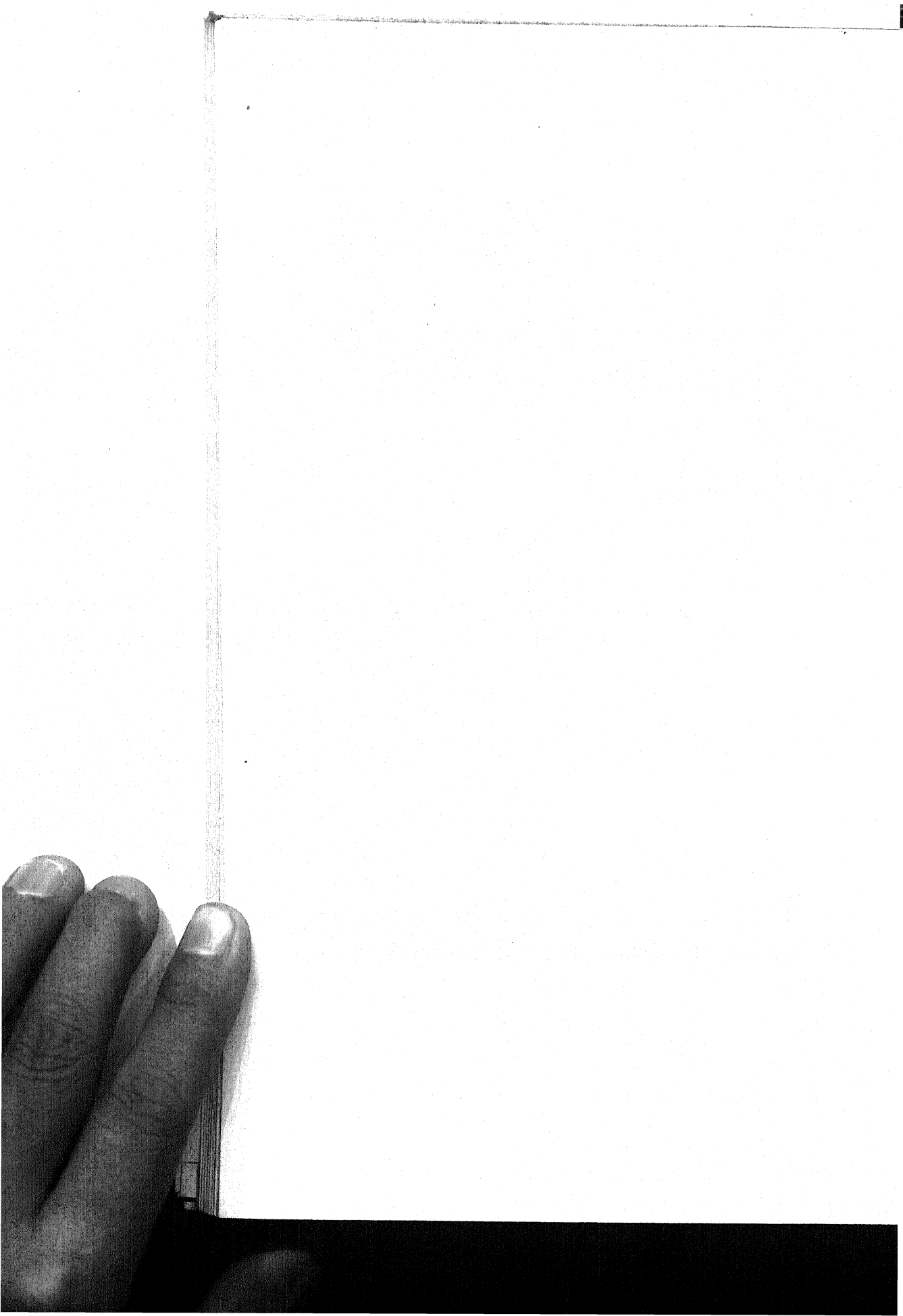


a b



c

McCREA: CLAVICEPS



EFFECTS OF MINERAL SALTS UPON THE TRANSPIRATION AND WATER REQUIREMENT OF THE COTTON PLANT[1]

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INTRODUCTION

Burgerstein (1904, 1920, 1925) has summarized most of the work on the effect of mineral salts upon the transpiration and water requirement of plants. In general the results of most of the earlier investigators agreed in showing that while transpiration increased in the presence of very small concentrations of most mineral salts as compared with the rate in distilled water, the rate decreased progressively with increase in concentration of the salts. The same general results were obtained with both soil and solution cultures, with single salt solutions, and with mixtures of salts or nutrient solutions.

The more recent investigations in this field which appear to have the most direct bearing upon the problem under consideration will be briefly reviewed here.

Harter (1908) grew wheat plants in soils containing .09 and .12 percent of total salts, and in garden loam. Of the soluble salts in these soils .06 and .08 percent, respectively, were estimated to be sodium chlorid. He found an increase in transpiration over the controls grown in garden loam for both of the soils containing an excess of salt; this increase was more marked for those plants grown in the less saline soil than for those grown in the more saline soil.

Reed (1910) concluded from the results of his experiments upon the effect of chemicals on transpiration that lime and sodium phosphate resulted in a material increase in transpiration, while potassium salts decreased it and sodium nitrate showed variable results. He believed these effects to be due to a specific action of the ions involved. He states: "Potassium always showed its inhibiting action on transpiration, regardless of whether it was combined with Cl, NO_3, or SO_4; so also was the accelerating action of calcium." It should be noted that Reed has presented his results in terms of "correlative transpiration" which he defines as the number of grams of water transpired for each gram of green substance produced. Furthermore, his investigation deals only with the effects of small amounts of salt upon the correlative transpiration of wheat plants. Sodium nitrate, potassium sulfate, and monocalcium phosphate were applied at a proportion

[1] Papers from the Department of Botany, the Ohio State University, No. 253. The experimental part of this investigation was performed at the Desert Laboratory of the Carnegie Institution of Washington at Tucson, Arizona, during the summer of 1929.

of 100 parts per million of soil, while calcium carbonate was supplied in the proportion of 1,000 parts per million. Hence this study does not furnish data concerning the effects of these several salts over any range of concentrations, but only at one very dilute concentration of each salt.

Bouyoucos (1911) carried out some extensive investigations on this problem. Wheat, corn and bean plants were grown in solution, sand, and soil cultures, in which the concentration of the nutrient solution varied from 0 to 4500 parts per million. In all three types of cultures it was shown that the transpiration for each unit of dry matter produced increased with increase in concentration of the nutrient solution up to a certain concentration, after which it decreased. Solutions of sodium chlorid and sodium sulfate, of the same range of concentrations as the nutrient solutions, showed essentially the same effects upon transpiration. It was believed by Bouyoucos that the increase in transpiration at low concentrations was due to a "stimulating" effect of the ions present upon transpiration, while the progressive decrease in transpiration after a certain concentration of solution is reached was due to osmotic retardation of the movement of water into the roots. When wheat seedlings were grown in $N/50$ solutions of various salts to each one of which was added the same amount of a nutrient solution the magnitude of the transpiration was quite different in the various solutions. It was believed therefore that the ions of these several salts have specific effects upon the rate of transpiration.

Hansteen-Cranner (1914) grew wheat, oats, and rye seedlings in normal Knop's nutrient solution for from two to seventeen days, after which they were transferred to dilute single-salt solutions and allowed to remain for four to fourteen days, the water absorption and the water loss for this period being measured. The salts used were generally potassium nitrate, sodium nitrate, and calcium nitrate. The sodium and potassium salts were usually employed at a concentration of $N/100$, while the calcium salt was employed at a concentration isosmotic with the other two salts. The calcium ion was found to exert a retarding influence on water absorption, and an accelerating effect on transpiration, while the potassium ion in isosmotic concentration stimulated water absorption and retarded transpiration. The sodium ion retarded both water absorption and transpiration. When the potassium and calcium ions were both present, both transpiration and water absorption were increased as compared with solutions in which only the potassium ion was present.

Kisser (1927) grew wheat plants in single-salt solutions for periods of about ten days and measured the water loss for that period, as well as the water absorbed, and the production of stem and roots. The wheat seedlings were allowed to develop for two weeks in a nutrient solution before being transferred to the salt solutions. The nitrates of calcium, magnesium, sodium, and potassium were employed in concentrations ranging from $M/50$ to $M/3200$. In general the nitrates of sodium, potassium, and magnesium

were found to depress transpiration as compared with distilled water while calcium nitrate accelerated it. The potassium salt usually showed an accelerating effect upon the absorption of water, while the other three salts usually diminished it. This work is thus in general agreement with that of Hansteen-Cranner. It should be noted that both of these investigators have dealt with very dilute concentrations of the salts employed, a procedure which reduces osmotic effects to a negligible point, and that both were largely concerned with specific ionic effects upon transpiration and water absorption.

Eaton (1927) has recently completed an investigation which involved the measurement of the effect of several concentrations of sodium chlorid applied to the soil upon the water requirement of the Australian salt bush (*Atriplex semibaccata* Brown) and wheat (*Triticum vulgare* Vell.). In these experiments the water content of the soil was maintained at approximately the moisture equivalent percentage. Sodium chlorid was added in amounts equivalent to .05, .10, and .25 percent of the dry weight of the soil. The water requirement of the Australian salt bush decreased progressively with increase in the salt concentration of soil, ranging from 352 for plants grown in soil to which no salt had been added down to 244 for plants grown in soil to which had been added .25 percent sodium chlorid. Addition of .05 percent of sodium chlorid increased the water requirement of wheat from 551 to 598. Larger additions of sodium chlorid decreased it, the values being 489 for .10 percent, and 354 for .25 percent of the soil. The concentration of the cell sap of these plants, measured in terms of freezing point depression or specific electric conductivity, was found to increase progressively with the increase in the concentration of sodium chlorid in the soil. In fact, with one exception in the case of the wheat the water requirement of these two plants was found to be inversely proportional to the concentration of the expressed cell sap, when these plants were grown on a soil to which sodium chlorid had been added in several concentrations.

The purpose of the present investigation was to determine some of the effects of adding relatively small quantities of mineral salts to a natural desert soil of low soluble salt content upon the rate of transpiration of plants growing in the soil. The salts employed were mostly among those found in the alkali soils of arid regions, and the range of concentrations applied corresponds roughly to the range of soluble salt concentrations in many soils which are used for agricultural purposes in such regions. These investigations were performed with cotton plants and except for the changes introduced by the varying percentages of salts in the soil, the plants in each series were all grown under the same external conditions.

Many of the previous studies of the effects of mineral salts upon transpiration, including some of those reviewed, have been concerned with the amount of water lost by plants over a period of time as referred to the production of a unit of fresh weight ("correlative transpiration"), or to a unit

of dry weight ("water requirement"). In the stricter usage of the term this is not an acceptable measure of the effect upon transpiration. Over a period of time the mineral salt concentration of the soil solution undoubtedly has effects, both direct and indirect, not only upon the water lost from the plant, but also upon the synthetic processes and growth of the plant. Results obtained from such experiments obviously cannot be interpreted entirely in terms of the effects of mineral salts upon transpiration. Since the present study is concerned primarily with modifications in the rate of transpiration as an individual process, the first part of this investigation is confined to short time experiments in which effects upon other processes should be relatively so unimportant that there is no reason to believe they will have any important bearing on the result.

Methods

This investigation was conducted at the Desert Laboratory, Tucson, Arizona during the summer of 1929. For the study of the effects of mineral salts on the rate of transpiration, weighed earthenware pots, 10.2 cm. in top inside diameter, were filled with a known weight (750 grams) of dry soil, watered with distilled water, and sown with several seeds of the Pima variety of cotton (*Gossypium* sp.). The soil employed was a coarse, well leached, sandy outwash, known to be of low soluble salt content.[2] This soil was screened through a 2 mm. mesh to remove coarse particles, and thoroughly air dried before the pots were filled. These pots were imbedded for about two-thirds of their depth in plots of the same soil. These plots were 1.83 meters square; each was covered with a wooden frame .91 meter in height, which was completely covered with a layer of fine mesh screen. A double layer of cheesecloth was tacked on the top and on the east, south, and west sides of these frames over the wire mesh in order to reduce the intensity of sunlight to which the plants were subjected. Otherwise the plants were exposed to normal outdoor conditions during their development. During the germination of the cotton seeds and the growth of the plants, the pots were watered with distilled water while the soil matrix was irrigated with water known to have a very low soluble salt content. A small amount of water was also provided at infrequent intervals by the brief showers typical at this season in Tucson. When the cotyledons became well developed the less thrifty plants were removed, leaving only one vigorous specimen in each pot.

For each series of determinations plants of the same history and age (about six weeks), and similar in general size and appearance were selected. The cotyledons were removed, leaving usually four well-developed leaves on each plant. The pots were removed from the beds, allowed to dry on the outside, brushed free from adhering lumps and particles of soil, and

[2] Measurements by the conductivity method, made by Dr. Forrest Shreve, showed the soluble salt content to be 44 parts per million, when equal parts of the soil and water were mixed.

fitted into 12.7 cm. Ganong aluminum shells. By weighing each shell and its contents it was possible to calculate very closely the amount of water which must be added to each pot in order to bring the soil in each to a 10 percent water content, which was slightly in excess of the determined moisture equivalent of 7.6 percent for this soil.[3] This calculation was made possible by the fact that the weights of the shell, pot, and dry soil were known, while the weight of the plant and of the water held in the walls of the pot could be estimated very closely from determinations on other pots of similar history. The amount to be added to the soil could thus be readily calculated from a weighing of the pot.

The calculated amount of distilled water into which had been introduced the requisite amount of salt for that pot from a standard solution was added to the soil of each pot. Series, each in duplicate, were run with sodium chlorid, calcium chlorid, potassium chlorid, sodium nitrate, calcium nitrate, and potassium nitrate. The first four of these salts, at least, are known to occur in considerable abundance in one or another of the numerous types of "alkali" soil common in the more arid parts of the United States (Harris, 1920). From four to six different concentrations of each salt, ranging from .05 to .8 percent of the dry weight of the soil, were employed in addition to the check pots for each series to which no salts were added. The calcium chlorid was employed in the mono-hydrate form, and the calcium nitrate in the tetra-hydrate form; all the other salts used were anhydrous.

As soon as the requisite amounts of water and dissolved salts had been added to each pot it was sealed to prevent evaporation of water from the soil or pot. A disk of felting paper cut to fit snugly against the wall of the pot and around the base of the plant was placed on top of the soil. Each pot was provided with a glass watering tube, which was fitted with a stopper. After the base of the stem of the plant had been protected with cotton and grafting wax, the entire top of the pot and the space between the wall of the pot and the wall of the shell were sealed over with a heavy layer of commercial "Plasteline." Finally a disk of tinned metal of the same diameter as the shell, through which apertures for the stem of the plant and watering tube had been cut, was placed on top of the Plasteline to protect it from dust and debris.

Each series was set up in the evening, the initial weighing being made about 9 P.M. After weighing, the pots were placed in the outdoor plots shaded by a double layer of cheesecloth. Weighings followed each evening for four days. The volume of water lost each day was restored after each weighing from a calibrated pipette using distilled water. At the end of the four day period each pot was dismantled and determinations of the final leaf area (initial leaf area having been determined when the experiments were set up), fresh weight of the top, and dry weight of the top were

[3] The writer is indebted to Mr. J. F. Breazeale of the U. S. Department of Agriculture, and the University of Arizona for placing at his disposal the equipment necessary for making these moisture equivalent determinations.

made for each plant. Transpiration for the four day period was then calculated on the basis of grams of water lost per square decimeter of the leaf area (the averages of initial and final leaf areas being used for this calculation), per gram of final fresh weight, and per gram of final dry weight.

For the accompanying study of the effects of sodium chlorid and calcium chlorid upon the water requirement of the cotton plant, large tinned buckets (17.8 cm. in diameter, 16.5 cm. high), provided with tight fitting lids were filled with 5500 grams of air dry soil of the same type as that used in the transpiration experiments. Sufficient distilled water, to which the requisite amount of salt had been added from a standard solution was added to each bucket to bring the water content of the soil up to 10 percent. The salt concentrations employed were equivalent to .0125, .025, and .05 percent of the dry weight of the soil for sodium chlorid and to .0125; .025, .05, .1, and .2 percent for calcium chlorid. Two four-day-old cotton plants were transplanted to each bucket from flats. Three 1-cm. holes were bored through each lid. These lids were set into place so that the plants projected through two of the holes. A layer of Plasteline was placed around the top of the inside wall of each bucket, making a water-tight seal between the lid and the bucket. In addition each lid was fastened firmly in position with a strip of one and one-half inch surgeon's adhesive plaster which sealed the junction between the lid and the bucket around its entire circumference. The stems of the plants were protected at the point where they projected through the holes in the lid with cotton and these openings were sealed with a layer of grafting wax superimposed on the cotton. The third hole through the lid was used for watering the plants and was provided with a tight fitting rubber stopper. This arrangement for the determination of the water requirement of the plants was checked for water loss and for a two day period this was found to be less than the experimental error in weighing.

Each series of water requirement experiments was run in duplicate. Both series of experiments with calcium chlorid were set up on July 23, one sodium chlorid series was begun on July 22, the duplicate series on July 28. The plants were grown under frames similar to those used in the transpiration experiments. Both plants lived to the termination of the experiment in every bucket except for one plant growing in the soil at a .2 percent calcium chlorid content. This plant, however, is included in the calculations. The cotyledons on every plant abscised prior to the termination of the experiments, and were not included in the final weighings of the plants. Otherwise, except for a few small holes eaten in a few of the leaves by insect larvae, all of the plants remained intact throughout the investigation. The duration of each water requirement experiment was fifty days. At the end of this period the heights of the cotton plants ranged from 20 to 50 cm. depending upon the treatment which the soil had received.

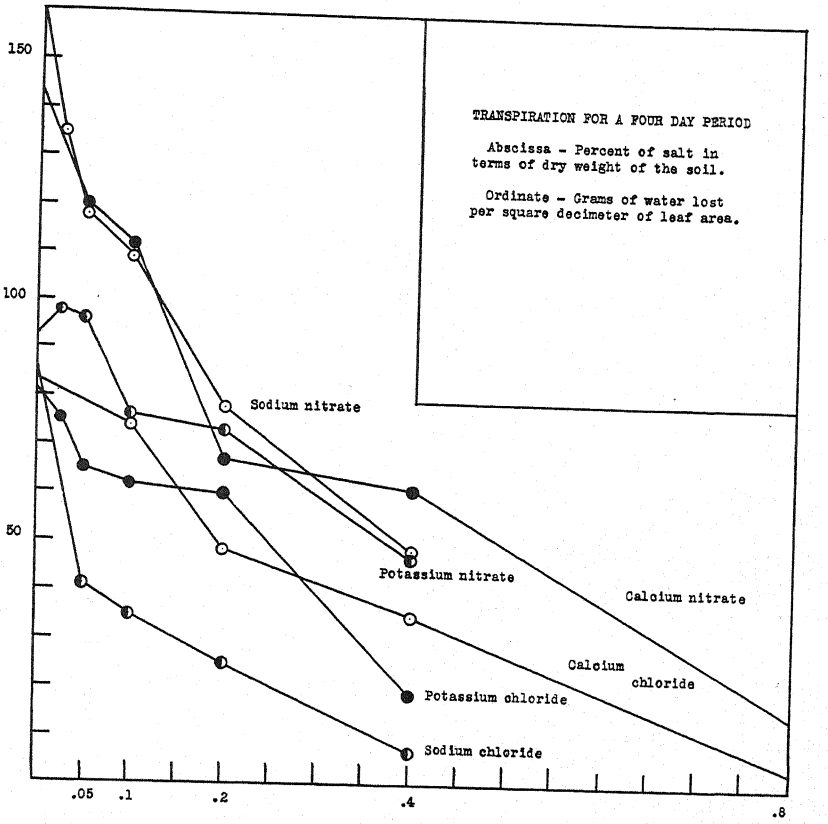
During the fifty day period the buckets were weighed on alternate days and the weight of water which had been lost by transpiration was restored

in units of 100 ml. from a calibrated flask. The level of the soil in the buckets was about 1 cm. below the top edge of the bucket. An air reservoir was thus provided between the soil level and the lid which assisted in the aëration of the roots, fresh air being introduced into the buckets each time the plants were watered. At the termination of the experiments when the plants were removed from the soil there was no indication of any kind that the root systems had suffered in any way from a deficiency of oxygen.

At the termination of the fifty day period the plants were cut off even with the tops of the lids and their fresh and dry weights determined. The water requirement of the cotton plants was then calculated for this period, this term being used in the sense that it was introduced by Briggs and Shantz (1913), and since generally used.

Results and Discussion

The results of the six series of experiments on transpiration are presented in two text figures. Text figure 1 represents the transpiration for each of the salt concentrations used in each of the series, based on one square decimeter of leaf surface. Text figure 2 represents the results of the same

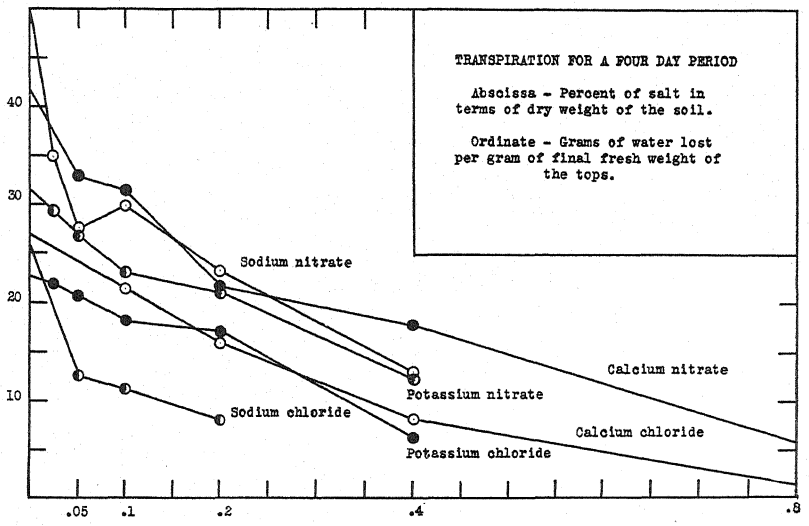


Text Fig. 1.

experiments with the transpiration calculated on the basis of each gram of fresh weight of the top at the termination of the four day experiments.

Inspection of these two figures indicates clearly that in general the addition of any of the salts employed in this investigation decreases the transpiration of cotton plants, and that there is a progressive reduction in the amount of transpiration as the percentage of salt applied to the soil is increased. No strict proportionality is shown between the magnitudes of transpiration and salt concentration, although for most of the salts employed there is some approach to such a relationship.

One minor irregularity is shown by the curve for sodium nitrate in text figure 2. In text figure 1 a slight increase in transpiration is shown for potassium nitrate in concentrations of .025 and .05 percent, otherwise all



TEXT FIG. 2.

results show a progressive lowering of transpiration with increasing concentrations of salts.

Other workers, as previously noted, have obtained slight increases in the transpiration of plants in salt solutions of very low concentration as compared with checks in distilled water. This result with potassium nitrate may be an isolated representative of this previously observed accelerating effect of very low concentrations of salts upon transpiration. In the present investigation check plants were grown in a natural soil of low salt concentration, and the study is primarily one of the effects upon transpiration of the addition of different concentrations of several salts to this soil. If accelerating effects are shown upon transpiration only by salt solutions in very dilute concentration they are probably largely masked in this investigation by the conditions under which the experiments were performed.

The concentration of the salts naturally present in the soil employed, low as it is, apparently is high enough to exceed the point where maximum accelerating effects are shown, with the one possible exception which has just been noted.

When the transpiration for the four day period was computed on the dry weight basis, the resulting graphs showed essentially the same trends as those plotted on the fresh weight basis, although the absolute values were, of course, different. Hence the results on the dry weight basis are not figured.

Since each experimental series began on a different date it is not possible to make a critical comparison of the effects of the several salts and ions involved. The possibility of making some approximate comparisons is not completely precluded, however. If the percent of transpiration at a given salt concentration, say .4 percent, be calculated with reference to the amount of transpiration in soils to which no salt was added for each series, mathematically comparable data result, which give an approximate conception of the effectiveness of the several salts employed in retarding transpiration. It is obvious that such calculated percentages are not strictly comparable, since they imply the probably unwarrantable assumption that retardation of transpiration will be proportionately the same when environmental conditions are favorable to a rapid rate of transpiration, as well as when they permit only a relatively slow rate. These data are therefore indicative only. On this basis the maximum retarding effect on transpiration at a concentration of .4 percent, considering both results calculated on a leaf area basis and those calculated on a fresh weight basis, is shown by sodium chlorid, followed in order by potassium chlorid, calcium chlorid, sodium nitrate, calcium nitrate, and potassium nitrate. The last two salts are almost exactly equal in effectiveness.

In general it may be stated that perhaps the most instructive conclusion to be drawn from text figures 1 and 2 is the marked retarding effect of relatively small quantities of salts upon the rate of transpiration. In this connection it should be recalled that the natural soluble salt content of this soil was low, and that the soil was maintained at the moisture equivalent percentage, which was considerably lower than that of most agricultural soils. The same quantities of salts, present in soils of higher water content, would not be expected to be so effective in reducing transpiration. In the present experiments addition of any of the salts employed in amounts equivalent to .1 percent of the dry weight of the soil or greater resulted in a distinct decrease in the amount of transpiration. At a .05 percent content in the soil, every salt except potassium nitrate caused a decrease in transpiration.

It is well known that the soluble salt content of many natural soils, particularly in the more arid regions of the world, equals or exceeds the concentrations used in these experiments. Large areas of such soils,

including even some of the so-called "alkali soils," are used for agricultural purposes, at least for certain crops. It seems probable that the principles regarding the effect of soluble salt content of the soil on transpiration, as elucidated by these experiments, would apply to most if not to all species of plants grown under these conditions.

Furthermore such a soluble salt content of the soil is sometimes exceeded in agricultural practices through the addition of mineral fertilizers to soils which are naturally of low soluble salt content. This is more likely to happen in the more intensive forms of agriculture as practiced in greenhouses and in truck gardening, and its occurrence is most common when fertilizer is so added as to have a cumulative effect on the soluble salt content of the soil. Cases are known where greenhouse soils have been rendered practically sterile for certain crops through excessive applications of fertilizer to the soil. An example of this is described by Conner and Gregory (1927). Such sterility is primarily due to "physiological drought" and is directly related to the investigation under discussion.

The fact seems to have been well established by other investigators, as previously noted, that very low soluble salt concentrations in a soil, at least for some salts or salt mixtures, may exert slight accelerating effects upon transpiration as compared with the rate in distilled water. However, it appears probable in the light of these experiments that under many natural conditions, and even some cultural conditions, the presence of soluble salts in the soil often results in a retardation rather than in an acceleration of transpiration. The concentration which results in maximum accelerating effects is undoubtedly frequently exceeded, and retardation of the transpiration rate is probably very common. Such an effect of mineral salts upon transpiration is undoubtedly of significance in many problems involving the water relations of plants, and would be expected to influence metabolic processes as well.

An analysis of the physiological mechanism whereby mineral salts influence the rate of transpiration was not planned as a part of the present investigation. It is known, however, that concentration of salts in the soil solution results in a retardation of the osmotic movement of water into the root hair cells. This retarding effect increases with increase in concentration, and according to the generally accepted conception of the movement of water through the plant would indirectly exert a retarding influence upon transpiration. As has been stated previously, the order of effectiveness of the salts employed in reducing transpiration at the .4 percent concentration was sodium chlorid, potassium chlorid, calcium chlorid, sodium nitrate, calcium nitrate, and potassium nitrate, the last two being equal in their effects. A calculation of the osmotic concentrations of these six salts shows that this is also the order which .4 percent solutions of these salts stand in respect to this property, with the one exception that the positions of potassium nitrate and calcium nitrate are interchanged. This result

appears to suggest that the differences in the retarding effects of these different salts upon transpiration in these experiments are due principally to the colligative properties of the solutions employed, rather than to any specific chemical effects of the various ions.

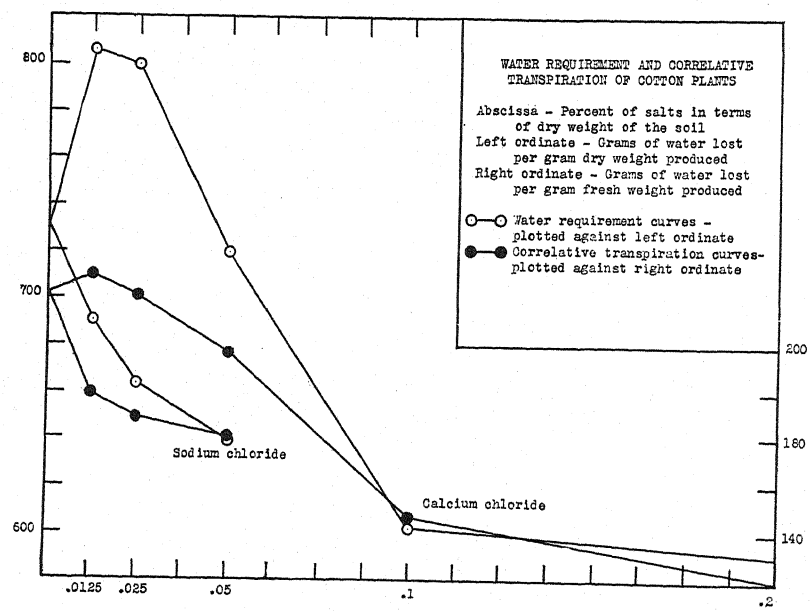
It would not, however, be expected that the retarding influence of different salts would necessarily be the same in magnitude, even if the salts were applied as solutions of equal osmotic concentration, because of difference in the "penetrability" of the various ions to the protoplasmic membranes. It is known that the calcium ion is much slower in penetrating protoplasmic membranes than the sodium or potassium ions (Scarth, 1925, and others). In short time experiments, such as most of those performed in the present investigation, this penetration differential among these three cations in all probability would have an influence on the rate of the osmotic movement of water into the roots, and hence on transpiration. The more rapidly the ion penetrates, the less effective it will be in retarding this movement of water. Closely related to this question is the problem of the effect of these several salts upon the permeability of the cell membranes to water. Clearly any such effects will have an important influence on the rate of movement of water into the peripheral cells of the root.

Because of the known complexity of the physical and chemical effects of mineral salts upon the physiological processes of plants, possibility of other less apparent but none the less important effects of salts upon transpiration should not be dismissed without mention. Among these are toxicity effects and that of mineral salts on the stomatal mechanism. Iljin (1922) reported that sodium and potassium salts stimulate the opening of the guard cells, while calcium salts check this process.

With the addition of the higher concentrations of sodium chlorid and calcium chlorid a marked tendency towards the abscission of leaves was observed. Actual abscission of leaves during the period of an experiment occurred in only one of the plants in the sodium chlorid series at a salt content of .4 percent. Two leaves abscised in three days from this plant. In the calcium chlorid series abscission of the leaves occurred from plants growing at the highest concentration of this salt on the day after the completion of this series. No abscission of the leaves occurred with any of the other salts employed, although a distinct drying of the lower leaves, especially at the edges, was observed with the highest concentrations of calcium nitrate and sodium nitrate. Neither drying nor abscission of leaves was apparent with either of the potassium salts employed, although the leaves of those plants treated with the highest concentration of potassium chlorid showed a distinctly lighter shade of green coloration at the end of the four day experiment as compared with the leaves on other plants in the same series, which were all bright green in color and turgescent (similar effects of the potassium ion were noted by Hansteen-Cranner, 1914). In a preliminary series of experiments, conducted to determine the maximum

tolerance of cotton plants to salts for short periods, abscission of leaves was induced by a soil content of .6 and .8 percent calcium chlorid and .4 percent sodium chlorid, but although 1.0 percent calcium chlorid and .6 percent sodium chlorid caused rapid wilting and drying of the leaves, no abscission took place within five days of the time the salts were applied to the soil. It is evident that in these plants that desiccation of the leaf tissues occurred so rapidly that the usual physiological changes associated with the dissolution of the abscission layer were inhibited, and hence abscission failed to occur. The causal factor in the abscission of leaves under the conditions just described is undoubtedly an internal drought condition resulting from the high osmotic concentration of the soil solution. The abscission layer in the petioles of herbaceous plants does not intercept the xylem tubes, and hence does not appreciably affect movement of water through them. Furthermore, rupture of the xylem elements is nearly the final step in abscission. Hence it seems improbable that there is any appreciable retardation of transpiration due to interference with water movement through the petiole during the incipient stages of abscission. Possibly there is a slight retarding effect during a short period just preceding leaf-fall. Interference with the movement of water through the other tissues of the petiole would begin just as soon as dissolution of the abscission layer started, but very little of the water which is lost in transpiration from the leaf blade moves by this route.

In text figure 3 are presented curves representing the effect of calcium



TEXT FIG. 3.

chlorid and sodium chlorid in different concentrations upon the number of grams of water required for the production of each gram of dry weight (water requirement) and for the production of each gram of fresh weight (correlative transpiration) for fifty-day-old cotton plants. The water requirement decreases rapidly with an increase in the concentration of sodium chlorid. This result agrees in general with those of Eaton (1927) who worked with wheat and Australian salt bush. The water required for the production of each gram of fresh weight also decreases rapidly at first, but more slowly with increasing concentration of sodium chlorid.

Addition of calcium chlorid results at first in a marked increase in the water requirement, followed by a rapid decrease at the higher concentrations. A curve similar in its salient aspects results when the number of grams of water required for the production of a gram of fresh weight in the cotton plant is plotted, except that the initial rise is relatively less prominent. As far as the lower concentrations of calcium are concerned these results agree with those of Reed (1910), who found that calcium had an accelerating effect when transpiration was measured in terms of fresh weight produced.

TABLE 1. *Percent Water Content of Tops of Cotton Plants Grown for 50 Days in Soil Containing Different Percentages of Salts*

Salt	Percent Salt on Basis of Dry Weight of the Soil					
	0	.0125	.025	.05	.1	.2
Sodium chlorid.....	66.1	71.1	72.3	72.4		
Calcium chlorid.....	66.1	69.3	69.6	73.0	75.8	79.9

One additional result of interest is illustrated in table 1, which shows the water contents of the tops of the cotton plants when harvested at the termination of the fifty-day experiment. These data are the averages for duplicate series. It is evident that when the cotton plants are grown in a soil of the type used in this investigation to which either sodium chlorid or calcium chlorid is added, there is an increase in the final water content of the tops and this increase is progressively greater with increase in the salt content of the soil. Thus it appears that one effect of the addition of mineral salts, at least within certain limits, to the soil in which cotton plants are growing is to increase the succulence of the tops of the plants. This fact probably has a bearing on some of the water relations problems of plants growing in soils relatively high in soluble salt content.

SUMMARY

A study has been made of the effects of several concentrations of certain mineral salts, applied individually, upon the transpiration and water requirement of cotton plants growing in a natural soil of low soluble salt

content. The soil was maintained at a water content of 10 percent, its determined moisture equivalent being 7.6 percent.

The salts employed for the transpiration experiments were sodium chlorid, sodium nitrate, potassium chlorid, potassium nitrate, calcium chlorid, and calcium nitrate. The first four were applied to the soil in concentrations of .025, .05, .1, .2, and .4 percent of the dry weight of the soil; with the calcium salts an additional concentration of .8 percent was used. For four-day periods addition of any of these salts in all the concentrations employed, with the exception of the two lowest concentrations of potassium nitrate, resulted in a decrease in transpiration as compared with plants growing in the soil to which no salt was added. Transpiration progressively decreased in amount with progressive increases in the concentration of any of these salts in the soil. The results were essentially the same whether transpiration was calculated on the basis of leaf area, fresh weight of the top, or dry weight of the top. It is clear that the concentrations of salts employed with reference to the soil conditions used lie in the range where osmotic effects predominate over specific ionic effects.

Sodium chlorid, in concentrations of .0125, .025, and .05 percent of the dry weight of the soil, and calcium chlorid in concentrations of .0125, .025, .05, .1, and .2 percent of the dry weight of the soil, were used in the water requirement study. Cotton plants were allowed to grow under these treatments for fifty days, the usual technique of water requirement studies being followed. The water requirement of the plants treated with sodium chlorid decreased progressively with increased concentration of this salt in the soil. The two lowest concentrations of calcium chlorid employed resulted in an increased water requirement; beyond that concentration the water requirement decreased progressively with increase in the concentration of calcium chlorid in the soil. Shifting the basis of calculation to the fresh weight basis ("correlative transpiration") instead of the dry weight basis did not alter the general trend of the results. A progressive increase in the concentration of sodium chlorid or calcium chlorid in the soil resulted in a progressive increase in the final water content of the tops (leaves and stems) of the cotton plants.

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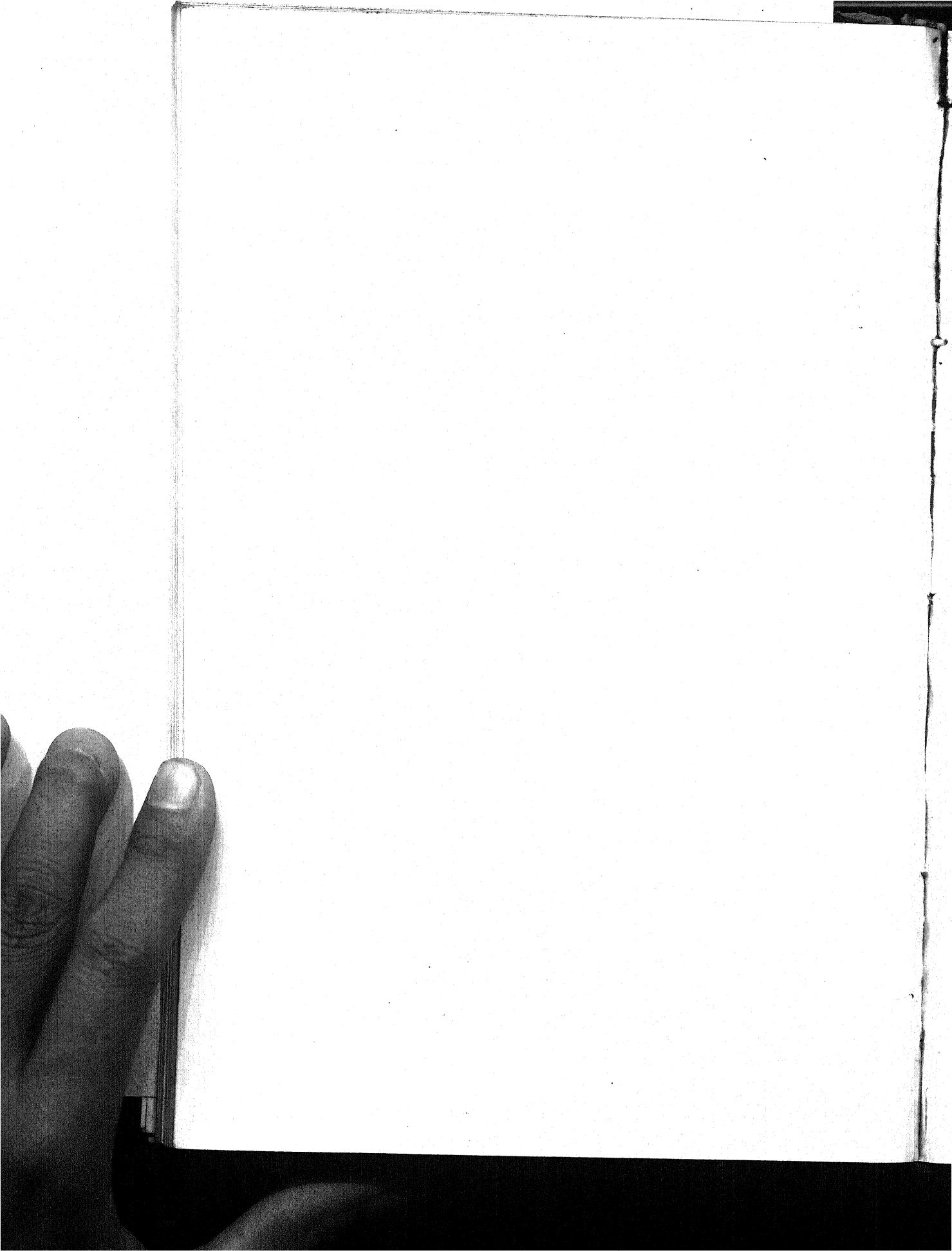
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PRELIMINARY STUDIES ON THE EFFECT OF FLOODING ON *PHYMATOTRICHUM* ROOT-ROT [1]

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Observations during the last twelve years in different sections of Texas have shown that root-rot, caused by the fungus *Phymatotrichum omnivorum* (Shear) Duggar, is extremely uncommon in creek or river bottoms which are subject to periodic overflows. In these bottom lands, including thousands of square miles of highly fertile land, usually planted to cotton, root-rot is absent; while 20 to 50 percent of cotton growing in adjoining uplands may be destroyed by root-rot year after year. This interesting condition has suggested the desirability of determining the cause of the absence of root-rot in bottom lands, with the idea that this information might be useful also in developing control methods that could be used in uplands.

One possible explanation for the absence of root-rot was that bottom-land soils might have become acid in reaction. Previous field surveys and inoculation experiments (3, 5) have shown that the disease is extremely destructive in neutral or alkaline soils, but is rarely important in acid soils. However, pH determinations of soils from several river-bottom lands showed them to be rich in calcium, and alkaline. Samples of Miller clay soil collected in Brazos County in the typical bottom along the Brazos River, where root-rot has never been found, gave a pH reading of 7.8 on the surface, becoming increasingly alkaline to pH 8.3 at 30-36 inches deep. This same type of soil, with similar reaction, occurs also in uplands in other counties; and root-rot is destructive on cotton grown in such places.

Since the soils in bottom lands are alkaline, an attempt was made (1) to inoculate plants growing in a typical bottom soil, to determine whether conditions other than soil reaction might operate to prevent the introduction of the disease.[2] A typical creek bottom, subject to frequent overflows, was

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[2] The writers are indebted to B. F. Dana, Plant Pathologist at the Blackland Substation, for supervising the inoculation of these plants and observations of the results.

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available at the former site of Substation No. 5. This land had been cropped to cotton and corn for a number of years, without root-rot ever appearing; yet cotton on uplands immediately adjoining this bottom suffered heavily from root-rot every year. During the summer of 1927, cotton plants in this bottom were artificially inoculated by the method previously described by us (4). The inoculated plants succumbed to root-rot. They were allowed to winter over, and root-rot reappeared on the cotton crop of the following year, spreading to many more plants than were infected in 1927. The normal soil conditions were therefore apparently not unfavorable for the introduction, spread, and overwintering of root-rot. Its general absence in bottom lands would thus appear to be due to the soil conditions during or immediately following the periods of inundation.

The Effect of Flooding on the Longevity of Strands on Infected Cotton Roots

An experiment was planned specifically to determine the effect of flooding on the longevity of the vegetative stage of the root-rot fungus, as it occurs on and in diseased roots. Naturally-infected tap-roots, from plants which had only recently wilted as a result of root-rot, were used. A large number of such plants were pulled on September 10, 1929, in a field of Crockett clay loam in Brazos County. Each of the 600 tap-roots selected for use in the experiment was cut down to a piece 10 to 14 inches long and the lateral roots were clipped off. Each of these 600 trimmed tap-roots included a lower portion decayed by root-rot, and also live, green tissue above the infected area. The roots were apportioned into three series of 200 each, kept moist over night, and stored thereafter as follows:

Series A. Roots Stored in Flooded Soil. Surface soil material of Crockett clay loam was used, in five-gallon crocks. This soil was obtained from the field in which the infected root material was collected, and while the pH was determined as about 6.2, the soil was favorable for the development of root-rot. In each of five crocks, four inches of soil was first placed on the bottom; the infected roots were then placed upright in the crocks, interspersed with soil; the tops of the roots covered with an additional two inches of soil; and the whole saturated with rain-water (from a cistern) and kept flooded until the end of the experiment. This series was run in the laboratory at room temperature, which averaged 26° C. on September 11, but dropped during October to a daily range of about 19° to 23° C.

Series B. Roots Stored in Moist Air in the Laboratory. Three inches of moist soil were first placed on the bottoms of the crocks; the roots were placed, upright, on the soil; and the crocks were then covered with moist burlap sacks, held in place by loosely-fitting plates.

Series C. Roots Stored in Moist Air at Low Temperature. The roots were arranged in the crocks as in Series B, and stored in the ice-storage room of the College ice-plant, at an average temperature of 1° to 2° C. These roots were not actually frozen, but were held at just above freezing.

Periodically, 20 of the infected tap-roots were withdrawn from each series, and the viability of *P. omnivorum* on and in these roots was tested in two ways. Of each set of 20 roots, 4 were placed in glass jars of moist Crockett clay loam soil, and the possible development of *P. omnivorum* strands along the walls of these soil-chambers was observed. It is to be noted that in observations on the soil-chambers, the presence of *Phymatotrichum* strands was recorded only when definite strands, identified microscopically, were produced next to the glass walls of the soil-chambers, or along the surface of the soil. Lack of growth was recorded, after the jars had been incubated in the laboratory for at least four weeks, only when there was still no trace of *Phymatotrichum* strand development to be found by careful examination.

The other 16 roots of each set were used to inoculate 10 to 12 cotton plants growing in two large metal cans during the early part of the season, or plants growing in large pots toward the end of the season. The inoculated plants were recorded as infected only when definite root-rot lesions were found on the roots, and presence of the fungus determined by microscopic examination. Plants that remained apparently normal were transferred in the fall to the greenhouse; the roots were washed out from the soil in January, 1930, and examined carefully. Repeated microscopic mounts were made from the roots of plants recorded as free from root-rot; these invariably showed no trace of *Phymatotrichum* strands.

At the times these roots were withdrawn from the various storage conditions and tested for virulence of the fungus, 20 additional roots were collected freshly in the field, and used in parallel soil-chamber and inoculation tests. These tests with freshly-collected roots are listed as Series *D*.

The results are given in table 1. Strand growth was obtained in the soil-chambers with roots after three days in the flooded soil (Series *A*), but not after a longer period of flooding, and none of the plants inoculated after the first day became infected. On the other hand, using roots stored in moist warm air (Series *B*) for two weeks, growth was still obtained and two of the thirteen plants inoculated with this material succumbed to root-rot. Roots stored in the ice-house (Series *C*) produced infection and strand growth after storage for three weeks. Refrigeration at just above freezing evidently increased the longevity of the fungus rather than injured its viability.

A further result of the experiment is obtained from comparison of the soil-chamber and inoculation data. It will be noted that these methods yielded almost identical results, in spite of the small number of soil-chambers used in each test. The occasional discrepancies between the results by the two methods are probably within the limits of experimental error. It would appear that the soil-chamber method of testing the viability of *P. omnivorum* can be considered a fairly reliable indication of the virulence of the inoculum also.

In this experiment, vegetative strands on and in roots stored in flooded soil were apparently killed in three to seven days, under temperature condi-

tions which allowed similar roots stored in moist air to maintain the fungus in a viable and virulent condition for at least two weeks. This suggests that the absence of root-rot in bottom lands which are subject to overflow may be due directly to the occasional or even rare destruction during periods of inundation of the active mycelium of the root-rot fungus on any infected roots that might be present.

TABLE 1. *Viability of P. omnivorum on Tap-roots of Naturally-infected Cotton Plants After Storage Under Various Conditions, as Demonstrated by Growth of Strands in Soil-chambers; and Virulence of Growth from These Roots as Shown by Inoculations to Normal Cotton Plants*

Date	Time Interval Since Experiment Started	Results of Tests *							
		A. Roots Stored in Flooded Soil, at Room Temperature		*B.* Roots Stored in Moist Air, at Room Temperature		*C.* Roots Stored in Moist Air, in the Icehouse at 1°–2° C.		*D.* Checks. Roots Collected Freshly in the Field at Each Date of Testing	
		Soil-chamber	Inoculation	Soil-chamber	Inoculation	Soil-chamber	Inoculation	Soil-chamber	Inoculation
1929									
Sept. 11	0 (initial)	+	+	+	+	+	+	+	+
" 12	1 day	−	+	+	+	+	+	...	...
" 14	3 days	+	−	+	+	+	+	+	+
" 18	1 week	−	−	−	+	+	+	+	+
" 25	2 weeks	−	−	+	+	+	−	+	+
Oct. 2	3 weeks	−	−	−	−	+	+	−	+
" 9	4 weeks	−	−	−	−	−	−	−	−
" 23	6 weeks	−	−	−	−	−	−	+	+
Nov. 6	8 weeks	−	−	−	−	−	−	+	−

* +, growth of strands in soil-chamber or successful inoculation; −, no growth or no infection.

FIELD EXPERIMENTS ON THE EFFECT OF FLOODING ON THE SURVIVAL OF ROOT-ROT [3]

Since *Phymatotrichum* strands on cotton roots in saturated soil appear to be inactivated so quickly, it should apparently be possible to control root-rot under field conditions by flooding the fields. Field experiments of this nature were carried out under irrigation conditions at Substation No. 16 at Iowa Park, Texas, during 1927, 1928, and 1929. The work was begun in 1927, in a field of Miller clay loam which had been in alfalfa since 1925, and in which root-rot had been very destructive. Early in the spring of 1927, the alfalfa was plowed under and the land divided into 6 plats each 40 × 80 feet. The plats were separated by borders 7 feet wide. Water secured from the main local irrigation ditch was applied to the plats, and maintained

[3] The writers are indebted to E. J. Wilson, Superintendent of the Iowa Park Substation, for assistance in these experiments. The water was furnished gratis by the Wichita County Water Improvement Districts No. 1 and 2; and Mr. G. A. Remington, General Manager, granted an uninterrupted flow of water during the periods of the experiments.

constantly at a two-inch level for definite intervals as indicated in table 2. After the flooding, when the soil had dried sufficiently, all the plats were planted on July 2, 1927, to cowpeas which are highly susceptible to root-rot. As noted in table 2, root-rot reappeared in all the plats whether flooded for 5, 10, 15, or 20 days. These periods of flooding, in early summer, apparently caused no reduction in root-rot as compared to the amount of infection in the check, unflooded plats.

TABLE 2. *Results of Flooding Experiment in 1927, on the Control of Root-rot on Cowpeas*

Plat Number	Time of Flooding	Number Days Flooded	Number Plants per Plat	Percent of Plants Killed by Root-rot
1	June 9 to 14, 1927	5	363	3.0
2	None	Check	388	4.4
3	June 9 to 19, 1927	10	378	3.4
4	June 9 to 24, 1927	15	480	5.0
5	None	Check	540	5.6
6	June 9 to 29, 1927	20	424	6.8

The work was repeated during 1928, when the floodings were carried out early in the spring. The field used had been in cotton, badly affected by root-rot in 1927. The land was plowed late in the fall of 1927, and was divided early the following spring into 6 plats each 40 × 80 feet and separated by 7-foot borders. As in 1927, water in each plat was maintained constantly at a two-inch level for definite intervals as shown in table 3. When the ground became sufficiently dry after the flooding, cotton was planted on April 24, 1928, in all 6 plats. Root-rot recurred in all plats, irrespective of the flooding (table 3).

TABLE 3. *Results of Flooding Experiment in 1928, on the Control of Root-rot on Cotton*

Plat Number	Time of Flooding	Number Days Flooded	Number Plants per Plat	Percent of Plants Killed by Root-rot
1	None	Check	491	3.5
2	March 13 to 18, 1928	5	325	6.2
3	March 13 to 23, 1928	10	182	1.7
4	March 13 to 28, 1928	15	245	5.7
5	March 13 to April 2, 1928	20	210	2.9
6	None	Check	297	3.0

During 1929, it was decided to continue the flooding experiment but for longer intervals. Another field was used in which root-rot had been very prevalent on the cotton grown in 1928. To insure the presence of much infected material uniformly distributed throughout this field, many of the plants were also artificially inoculated during 1928 with live cotton-root inoculum. The cotton was plowed under late that fall, and the field divided into 6 plats, each 40 × 90 feet, with a border 6 feet wide separating the

plats. As in 1927 and 1928, water was applied to the plats and maintained constantly at a two-inch level, but for the longer intervals indicated in table 4. Root-rot reappeared even in the plat which had been flooded for 120 days. The differences between the loss from root-rot in the various plats apparently were not associated with the differences in flooding.

TABLE 4. *Results of Long-time Flooding Experiment in 1929, on the Control of Root-rot on Cotton*

Plat Number	Time of Flooding	Number of Days Flooded	Number Plants per Plat	Percent of Plants Killed by Root-rot
1	None	Check	1172	3.8
2	November 15 to December 15, 1928	30	1050	3.0
3	February 15 to March 15, 1929	30	2906	0.5
4	November 15, 1928 to January 15, 1929	60	2561	1.4
5	November 15 to December 15, 1928 and January 15 to February 15, 1929	60	3519	8.2
6	November 15, 1928 to March 15, 1929	120	2113	4.8

Excavations were made in the plats and borders to determine to what extent the flooding had killed the roots of cotton and other plants left from previous years. On April 22, 1929, live cotton roots were found in all the plats, apparently irrespective of the flooding. There were also many live roots of a susceptible weed, *Solanum elaeagnifolium* Cav., which survived both in the plats and in the borders separating the plats.

DISCUSSION

Under the conditions of the experiments at Iowa Park, flooding continued even for 120 days did not eradicate root-rot nor kill out the roots of plants susceptible to root-rot. These results, obtained under field conditions, do not agree with the laboratory experiment, presented above, in which root-rot strands were inactivated by flooding for more than three days. It is, of course, possible that water standing on the surface of the plats under irrigation did not penetrate so thoroughly as to saturate the soil at a great enough depth to influence the survival of root-rot. Furthermore, King has shown (2) that sclerotia of *P. omnivorum* are still viable after being immersed in water for 121 days. This would explain the failure of a single long period of flooding to eradicate root-rot, since the period was apparently not long enough to have killed all the sclerotia even had the soil become completely saturated. It may be suggested that in the bottom lands, the encroachment of root-rot is prevented not necessarily by instantaneous eradication in overflowed areas but perhaps by the gradual diminution of infective material as a

result of recurrent floods. Studies on this problem are being continued both in the laboratory and in the field.

Summary

Root-rot caused by *Phymatotrichum omnivorum* is extremely uncommon in bottom lands, in soils apparently favorable for the development of the disease, even in regions in which the disease is prevalent in the adjoining fields. Root-rot was introduced into a creek bottom by the artificial inoculation of cotton plants, and reappeared in the year following the inoculation. In a laboratory experiment, strands of *P. omnivorum* on naturally-infected cotton roots were inactivated by submergence in saturated soil for more than three days; while with parallel series, at the same temperature but stored in moist air, the fungus was still viable after two weeks and still able to infect normal cotton plants.

Flooding experiments in the field, during three seasons and continuing for as long as 120 days, failed to produce significant changes in the survival of root-rot or to eliminate roots of root-rot carriers from the soil. The survival of root-rot in these experiments is explained as due to possible lack of deep penetration of the water, and to the possible presence of sclerotia which are able to survive long periods of immersion.

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STUDIES ON THE SENSITIVITY OF *MIMOSA PUDICA* I. THE EFFECT OF CERTAIN ANIMAL ANESTHETICS UPON SLEEP MOVEMENTS[1]

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The movements of *Mimosa pudica* have long puzzled observers of them and have been the subject of numerous investigations, and yet one finds, upon close examination of the literature, that data upon many of the fundamental factors which may affect or modify the intensity or type of response of this species are entirely lacking or even inaccurate and misleading. This condition was brought to my attention in connection with studies upon the comparative effects of various animal anesthetics on the movements of this plant. I have since extended the scope of these investigations and am now able to present a more or less complete series of data upon the more important factors involved.

The present paper is the first of a series and is limited to results obtained upon the effect of various animal anesthetics on the sleep movements of *Mimosa pudica*. The members of this series might be given in any order, but to simplify the description of experimental methods, I shall present them in the order of increasing complexity of method.

That the leaves of many plants assume a position during the night which is different from their characteristic day position has been known since the sixteenth century. The early discovery and general characterization of this phenomenon were well expressed by De Candolle (1832) in the following manner: "Val Cordus was first (1581) to observe the sleep of leaves of *Glycyrrhiza*, and Garcias de Horto (1567) noted that in India the leaves of *Tamarindus* closed together in the evening and reopened in the morning. One notices that many plants possess a similar faculty, and Linnaeus in his usual poetical style gave to this phenomenon the name of 'sommeil des feuilles.' It is necessary to remark, however, that this term does not convey just the same meaning in the two kingdoms. In the animal it always represents a state of rest or flaccidity of muscles and joints; while in the plant it indicates merely a change in position, but the night and day positions are accompanied by the same degree of rigidity and regularity of occurrence."

The general movements which *Mimosa pudica* exhibits in response to light or darkness are according to Mayo (1827) as follows: "In the daytime

[1] This paper is the first of a series of three, the other members of which will appear in subsequent issues of the Journal. This series constitutes a portion of the work done while the author was a National Research Council Fellow in Botany.

the petioles are observed to have an upward direction or rather to form an acute angle with the upper part of the stem or branch to which they are attached: the subleaflets are spread out, so as to lie nearly in one plane. During the night the petioles are found to be depressed; the subpetioles are drawn together, the subleaflets are folded, the upper solar surfaces of each pair being brought into contact."

Materials and Methods

All experiments reported in this series of papers were made upon vigorous young plants. Preliminary experiments demonstrated clearly that it is essential to use the best plants obtainable, for otherwise the results are variable and may perhaps lead to erroneous conclusions. It seems quite probable that many of the present inaccurate statements in the literature and in text-books may have been due to observations on poor plant material.

Excellent plants can be grown in the greenhouse from May to September, but during the rest of the year it is necessary to use artificial illumination or to supplement natural illumination. This use of artificial illumination, together with the details of the method, will be described fully in the last paper of this series. This species requires very strong light and high temperatures, from 30° to 40° C., for its maximum growth and sensitivity, but is relatively independent of moisture conditions. It will grow equally well in high humidity and with high soil moisture content, or in low humidity and with low soil moisture content, light and temperature being constant. The present experiments were made in the greenhouse during the months of July, August, and September, when conditions were very favorable to the growth of *Mimosa pudica*.

Most of the plants were from five to seven weeks old and had from four to six mature leaves. I have, however, tested seedlings only a week old and mature plants more than a year old and have found no specific differences in their reactions. This was probably to be expected in view of the fact that under normal growth conditions the plant renews its set of leaves every few weeks.

Plants were placed in twenty-liter belljars inverted in pots of sand and sealed with ground-glass plates in the manner described in my paper upon the effect of ethylene on woody twigs (1926). One-half of the interior surface of each belljar was lined with a piece of filter paper. The substance whose effect was to be tested was allowed to flow on this filter paper from the pipette in which it had been measured.

The required vapor concentration of each liquid substance was made up on the basis of the molar-volume-gas law, that one molecular weight of any liquid when converted to its vapor at standard temperature and pressure will occupy 22.4 liters. It is thus simple to calculate the weight of liquid which is required to give a one percent concentration at a temperature of 0° C. and a pressure of 760 mm. This value was then corrected from standard temper-

ature of 0° C. to 30° C., which is more nearly that of the experiments. It is to be assumed, therefore, that all concentrations of vapor are made up with 760 mm. pressure and 30° C. temperature as the arbitrary standards. These values will vary slightly from the actual values for each specific experiment, but as can be readily calculated the error is less than one percent and therefore probably negligible. On the other hand there is the very great advantage that the fixed standard makes possible the exact duplication of the experiments.

The volume of the compound whose required weight has just been determined is calculated by dividing that weight by the specific gravity of the substance in question. The next paper will include a table showing the number of cubic centimeters of each compound necessary to give a vapor concentration of one percent in a chamber with a capacity of twenty liters. Those concentrations in which the volume of liquid used was less than one cc. were measured in a pipette calibrated to .01 cc., while those greater than this were measured in a 10 cc. pipette calibrated to .1 cc.

The concentration in those experiments in which the gases, nitrous oxid, ethylene, and acetylene were used were made up by water displacement in a pneumatic trough, the plant itself being enclosed before the belljar was removed from the trough. The jar was then sealed with a glass plate and the experiment continued in the same manner as in those using liquids.

Results

The sleep movements of *Mimosa pudica* and at least two other species of plants can be prevented without permanent injury by certain concentrations of ether and acetylene. These plants were placed in the belljars in the presence of the desired compounds in the afternoon while the leaves and petioles of the plants were still in the normal day position. Several hours later, one hour after the control plants as well as the other plants of the same species in the greenhouse were in their usual night position, the experimental plants were examined and found to be still entirely open, partially open, or entirely closed, depending upon the concentration and kind of compound used.

Effect of Ether

It is very easy to prevent the evening closing or morning opening of the leaves of *Mimosa* by means of ether. One such experiment which may be taken as typical is given in table 1. Eight fresh, young, vigorous plants, each having four mature leaves, were placed in belljars and exposed to 5, 7, 9, 11, 13, 15, 17, and 19 percent ether, respectively, at 5 P. M. At 8 P. M., three hours later, leaflets of all *Mimosa* plants in the greenhouse regardless of age were fully closed and their petioles depressed. The leaflets and petioles of the experimental plants in the 5 percent ether were in the same condition, those in the 7 percent were just partially closed, and those in all higher concentrations were still fully expanded as at 5 P. M.

TABLE 1. *Effect of Ether and Various Other Compounds upon the Sleep Movements of Mimosa pudica* *

Compound		Sensitivity of Plant	Night Closing			Sensitivity of Plant	Morning Opening		
			Number of Leaves				Number of Leaves		
Name	%		Total	Open	Closed		Total	Open	Closed
Ether	5	+	6	0	6	+	5	5	0
	7	+	4	all 9/10 closed		+	4	4	0
	9	+	5	all ½ closed		+	4	4	0
	11	+	4	4	0	+	5	5	0
	13	−	3	3	0	−	5	all ½ closed	
	15	−	4	4	0	−	5	all ¾ closed	
	17	−	5	5	0	−	5	0	5
	19	−	5	5	0	−	5	0	5
C_2H_2	100	−	5	5	0	−	5	0	5
	98	+	4	all ¾ closed		+	4	all ¾ closed	
	95	+	6	0	6	+	6	6	0
C_2H_4	100	+	4	0	4	+	5	5	0
N_2O	100	+	4	0	4	+	5	5	0
$CHCl_3$	2	+	5	0	5	+	6	6	0

* Temperature for tables 1, 2, and 3: night closing, 80°–90° F.; morning opening, 65°–75° F.

On testing the effect of the ether upon the morning opening of the leaflets, however, one finds that several of the concentrations which were effective in preventing their evening closing are now apparently without effect (table 1). In fact, no concentration lower than 17 percent will completely prevent their opening, while concentrations of 13 and 15 percent will only partially prevent it. The leaflets continue to open and assume their day position in concentrations up to and including the 11 percent. It is quite evident from these data that while one can prevent either the evening closing or morning opening of the leaflets, the concentrations necessary are not alike. This is of special interest in view of the fact that other species, *Oxalis stricta* and *Marsilia macropus*, may show just the reverse relation with respect to concentration; that is, they can close in concentrations which will completely prevent their opening.

Perhaps the most interesting point which has come out of these studies is the beautiful demonstration, in the case of *Mimosa pudica*, that the ether has actually prevented the occurrence of evening closing, and that this reaction must be completed before the leaflets can assume their normal night position. Thus, when one removes a plant at 8 P. M. from a belljar in which the concentration had been 13 percent or greater, the pulvini of both the leaflets and primary petioles are anesthetized and hence will not respond to shock. After one to three minutes, however, the pulvini of the leaflets have regained their sensitivity and the pairs of leaflets will close together in response to stimulation. They will not remain closed, however, but will quickly return to their former completely expanded position. They can

then be caused to close again with like results. It will be observed, however, that after this has been repeated several times the leaflets are gradually assuming their night position, and open less and less after each closing. At the end of an hour they are usually entirely closed. This same condition doubtless holds true for the other pulvini as well. It has not been possible to demonstrate it, however, because it takes from 30 minutes to an hour for them to recover from the anesthetization, and this is sufficient time for the night closing to occur.

While I do not wish to discuss anesthesia at this time, in view of the fact that I have used the term in the above paragraph it is perhaps well to state that it is here used to mean complete loss of sensitivity and subsequent complete recovery with no injury. It will be observed in table 1 that 13 percent ether is the minimal concentration which will bring this about. A concentration above 22 percent is usually lethal.

The fundamental facts concerning the prevention of sleep movements are summarized by Plate IX. Plant *B* is the control and shows the normal position of leaflets and petioles at various times of day for *Mimosa pudica*. Plant *A* is the experimental plant and shows the prevention of the evening closing and morning opening of the leaflets. Plant *A* was photographed at 5 P. M. and then placed in ether (15 percent). When removed and photographed three hours later the leaflets and petioles were still in the 5 P. M. position. At 5 A. M., after being in the open air of the greenhouse for nine hours, the plant had recovered from the ether and its condition appeared identical with that of the control. It was then placed in 19 percent ether to prevent the morning opening. When removed and photographed three hours later (8 A. M.) the leaves and petioles were still in the 5 A. M. position. Detailed quantitative data of the actual angular position of the primary petioles for various times of day will be presented in the third paper of this series.

Effect of Chloroform

In direct contrast with the great ease with which one can prevent the sleep movements of *Mimosa* by means of ether is the impossibility of preventing them with the other common animal anesthetic, chloroform. Preliminary experiments established that the lethal concentration of chloroform for the foliar portions of the plant was about 2.5 percent, and even lower concentrations resulted in injury after long exposures. Since exposure times of at least several hours were necessary in tests upon sleep movements it was useless to attempt to use concentrations greater than 2 percent. As can be seen in the table this concentration was without effect on either the evening closing or morning opening. It will also be observed that this concentration of chloroform seemed to have little or no effect upon the sensitivity of the plant in response to shock. This discrepancy between the effects of ether and chloroform will come out even more clearly in connection with data to be presented in the next paper.

Effect of Gaseous Anesthetics

A similar series of tests with nitrous oxid, ethylene, and acetylene gave unexpected results. Nitrous oxid and ethylene, even in concentrations of 100 percent, have no effect whatever on sleep movements. Acetylene on the contrary, when in 100 percent concentration, gave results similar to those for the higher concentrations of ether. Ninety-eight percent acetylene, however, only partially prevented the evening closing and morning opening. A concentration of 95 percent had no apparent effect.

The question arises whether a slight impurity such as oxygen present in the cylinders of compressed gases may not explain the failure to obtain positive results with these compounds. No oxygen was found, however, in samples of these gases which were analyzed. Moreover *Mimosa pudica* is reported by Correns (1892) to retain its sensitivity in an atmosphere where the oxygen pressure has been reduced to less than 1/20 to 1/40 normal. It is perhaps well to state also that the loss of sensitivity to touch in the presence of 100 percent acetylene occurs in from 1 to 5 minutes while in the presence of ether it requires from 20 to 45 minutes, indicating a positive action on the part of the acetylene and not merely the effect of lack of oxygen. I realize, of course, that the possibility of a slight amount of oxygen being the basis of the failure to obtain similar results with nitrous oxid and ethylene has not been entirely eliminated. In view of the great mass of recent data relative to the effects of minute amounts of certain substances upon plant processes and reactions one must be very careful in accepting older less critical data as a basis for conclusions.

A very interesting point with respect to the gaseous anesthetics tested is that they appear to be entirely harmless to *Mimosa* if the temperature during the exposure is 30° C. or above. On the other hand, if the temperature is allowed to drop to 20° C. or below, the plants in acetylene and ethylene (but not those in nitrous oxid) will be entirely defoliated within a few hours. The detached leaflets are entirely normal in appearance for twenty-four hours or so after they have fallen.

Other Species

Oxalis stricta. That the results described above are not restricted to *Mimosa* alone but are probably characteristic for sleep movements of many plants is shown by tests made on two other species, *Oxalis stricta* and *Marsilia macropus*. The data on the former are given in table 2. It will be observed that the evening closing of this plant is inhibited by 13 percent ether, while a concentration of only 11 percent will prevent its morning opening. The gases have exactly the same effect or lack of effect, as the case may be, on the sleep movements of *Oxalis* as they did on *Mimosa*. It would seem, however, that one of the gases, acetylene, is slightly toxic to this species, since a few of the older leaves of the plant would often turn a peculiar brick red color some time after the plant was removed from the gas.

TABLE 2. *Effect of Ether and Various Other Compounds upon the Sleep Movements of Oxalis stricta*

Compound		Night Closing			Morning Opening		
		Number of Leaves			Number of Leaves		
Name	%	Total	Open	Closed	Total	Open	Closed
Ether	5	20	0	20	15	15	0
	7	18	0	18	12	12	0
	9	18	0	18	26	all ¾ closed	
	11	12	0	12	30	0	30
	13	25	all ¾ closed		19	0	19
	15	17	17	0	17	0	17
	17	11	11	0	15	0	15
	19	12	12	0	20	0	20
C_2H_2	100	14	14	0	14	0	14
	98	18	all ½ closed		18	all ½ closed	
	95	20	0	20	20	20	0
C_2H_4	100	20	0	20	20	20	0
N_2O	100	20	0	20	20	20	0
$CHCl_3$	2	17	0	17	20	20	0

Marsilia macropus. In like manner tests have shown that one can prevent the sleep movements of *Marsilia macropus* by certain concentrations of ether and acetylene (table 3). There is a great difference, however, in the concentrations of ether necessary to inhibit the evening and morning responses. Thus a concentration of 19 percent is required to prevent the leaflets closing together in the evening, while a concentration of only 9 percent will prevent them from reopening in the morning. The gases have the

TABLE 3. *Effect of Ether and Various Other Compounds upon the Sleep Movements of Marsilia macropus*

Compound		Night Closing			Morning Opening		
		Number of Leaves			Number of Leaves		
Name	%	Total	Open	Closed	Total	Open	Closed
Ether	5	5	0	5	4	4	0
	7	7	0	7	3	all ½ closed	
	9	13	0	13	8	0	8
	11	8	0	8	4	0	4
	13	4	all ¾ closed		4	0	4
	15	5	all ¾ closed		6	0	6
	17	7	all ¾ closed		9	0	9
	19	3	3	0	7	0	7
C_2H_2	100	6	6	0	6	0	6
	98	6	all ½ closed		6	all ½ closed	
	95	5	0	5	5	5	0
C_2H_4	100	9	0	9	9	9	0
N_2O	100	10	0	10	8	8	0
$CHCl_3$	2	8	0	8	7	7	0

same effect as in the former cases. The 100 percent partially inhibits the reaction, and 95 percent has no effect. As before, nitrous oxid and ethylene have no effect. Further there was little or no evidence of injury to this species by any of the gases tested.

Discussion

I have been able to find no data with reference to the effects of animal anesthetics upon the sleep movements of plants, with the exception of Pfeffer's general statement (1906) that "the repeated shaking of *Mimosa pudica* causes the suspension of seismonic irritability alone, as also do low temperatures and anesthetization, whereas the autonomic and daily movements continue." Upon what data Pfeffer based this conclusion it is impossible to determine. If it were upon tests with chloroform, carelessly used, the statement is entirely true. With ether, however, it is very obvious that such is not the case, but that instead one can very easily prevent either the evening closing or morning opening by using the concentration necessary.

This unexpected difference in the effects of ether and chloroform has led Correns (1892) to question whether those compounds which anesthetize animals will likewise anesthetize plants. He found that nitrous oxid, although it anesthetized animals, failed to insensitize the stamens of barberry. It seems very obvious from the data given above, as well as from additional data which will be presented in a later paper, that chloroform, ethylene, and some other animal anesthetics do not affect the plant in the manner in which they affect higher animals.

From the evidence given above ether is the only compound of the five tested in these experiments which can be considered as giving effects analogous to those obtained with animals. The single compound, acetylene, which gives a similar effect, must be present in such high concentration (98–100 percent) as to practically remove it from the realm of anesthetics. The other two gases are apparently without effect however strong the concentration. It is not at all impossible if one could find a plant which could stand a higher concentration of chloroform than *Mimosa pudica* and the other species tested that sleep movements might be inhibited. That chloroform is a more dangerous anesthetic to use is well known in medical practice and my results abundantly confirm this.

I called attention (1928) to the fact that from the morphogenetic viewpoint the intumescences which will develop in the stems and buds of many woody plants in response to ethylene gas were very similar to the formation of abscission layers as reported by Lloyd (1916). The present work has brough out another point which is even more striking evidence of a similarity in the two phenomena. One can expose *Mimosa* plants repeatedly to any desired concentration of ethylene or acetylene so long as the temperature remains above 30° C., and there will be no evidence of injury. If, on the other hand, the temperature is allowed to remain at 20° C. or lower,

complete defoliation occurs in a few hours. The primary petioles separate from the stem, the secondary separate from the primary, and the leaflets in turn separate from the secondary. This inhibition of response by high temperature and its increase at low temperature are the same as I found to exist in the development of intumescences in response to ethylene gas. No intumescences would form at or above 30° C., but at 20° or less they formed readily.

Summary 1

1. Careful quantitative experiments have shown that the sleep movements of *Mimosa pudica*, *Oxalis stricta*, and *Marsilia macropus* can be inhibited by certain concentrations of ether and acetylene. The concentration of ether necessary to prevent the evening closing is different from that necessary to prevent the morning opening in the three species.

2. The lethal concentration (about 2.5 percent) of chloroform seems to have little or no effect on the sleep movements of the three species studied.

3. The plants continue to show their usual evening closing and morning opening in atmospheres composed of approximately 100 percent nitrous oxid or ethylene.

4. Very high concentrations of acetylene, ethylene, and nitrous oxid are harmless to the foliage of the three species.

All data given in this series of papers were obtained in the Columbia University laboratories and greenhouses. I feel very grateful to Professor S. F. Trelease for the unrestricted use of this equipment. It is with pleasure that I express appreciation to Professor R. A. Harper for his kindly interest and his many helpful suggestions in this study.

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EXPLANATION OF PLATE IX

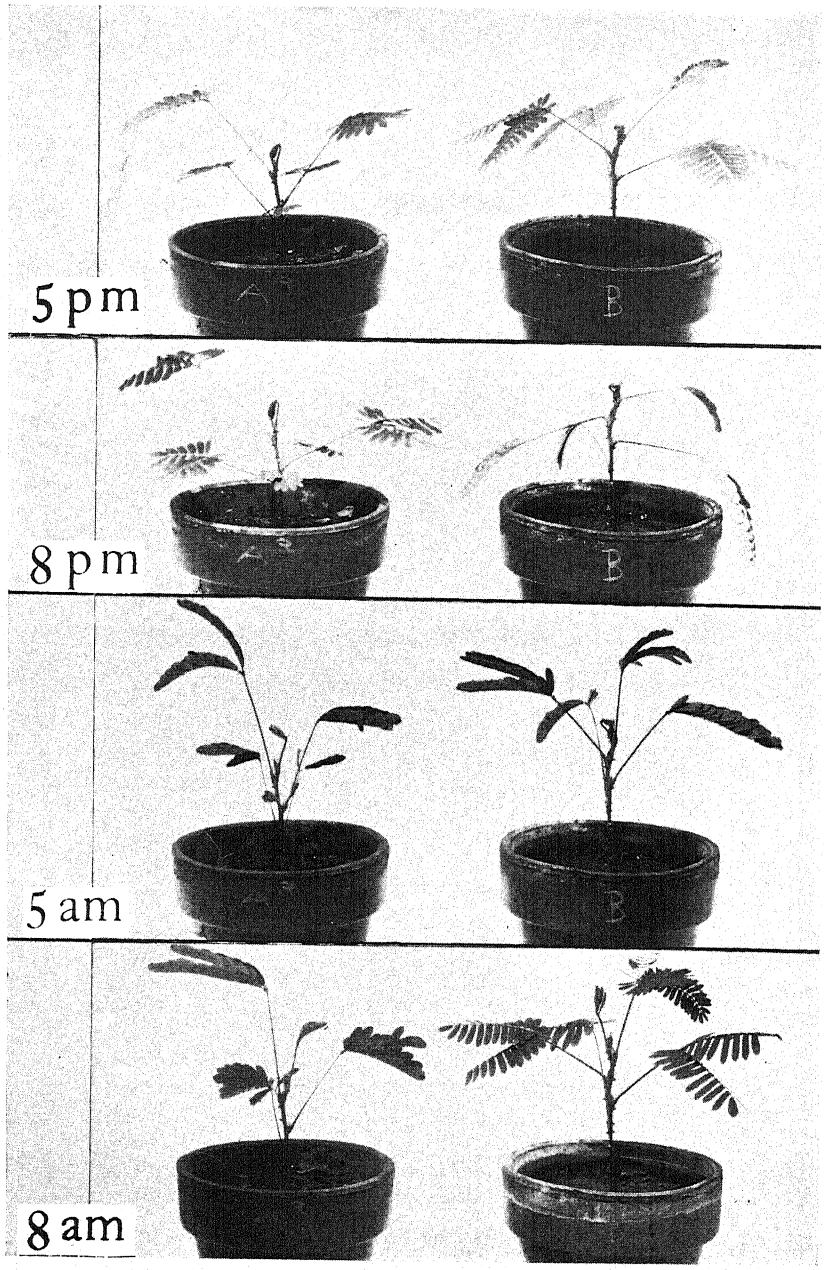
Photographs showing the prevention of the sleep movements of *Mimosa pudica*. Plant *B* (right) is the control and shows the normal position of the leaflets and petioles at 5 P.M., 8 P.M., 5 A.M., and 8 A.M. rsepectively. Plant *A* (left) is the experimental plant and shows the prevention of the evening closing and morning opening of the leaflets. Plant *A* was photographed at 5 P.M. and then placed in ether (15 percent). When removed and photographed three hours later the leaflets and petioles were still in the 5 P.M. position. At 5 A.M., after being in the open air of the greenhouse for nine hours, the plant had recovered from the ether and appeared identical with the control. It was then placed in 19 percent ether to prevent the morning opening. When removed and photographed three hours later (8 A.M.) the leaves and petioles were still in the 5 A.M. position.

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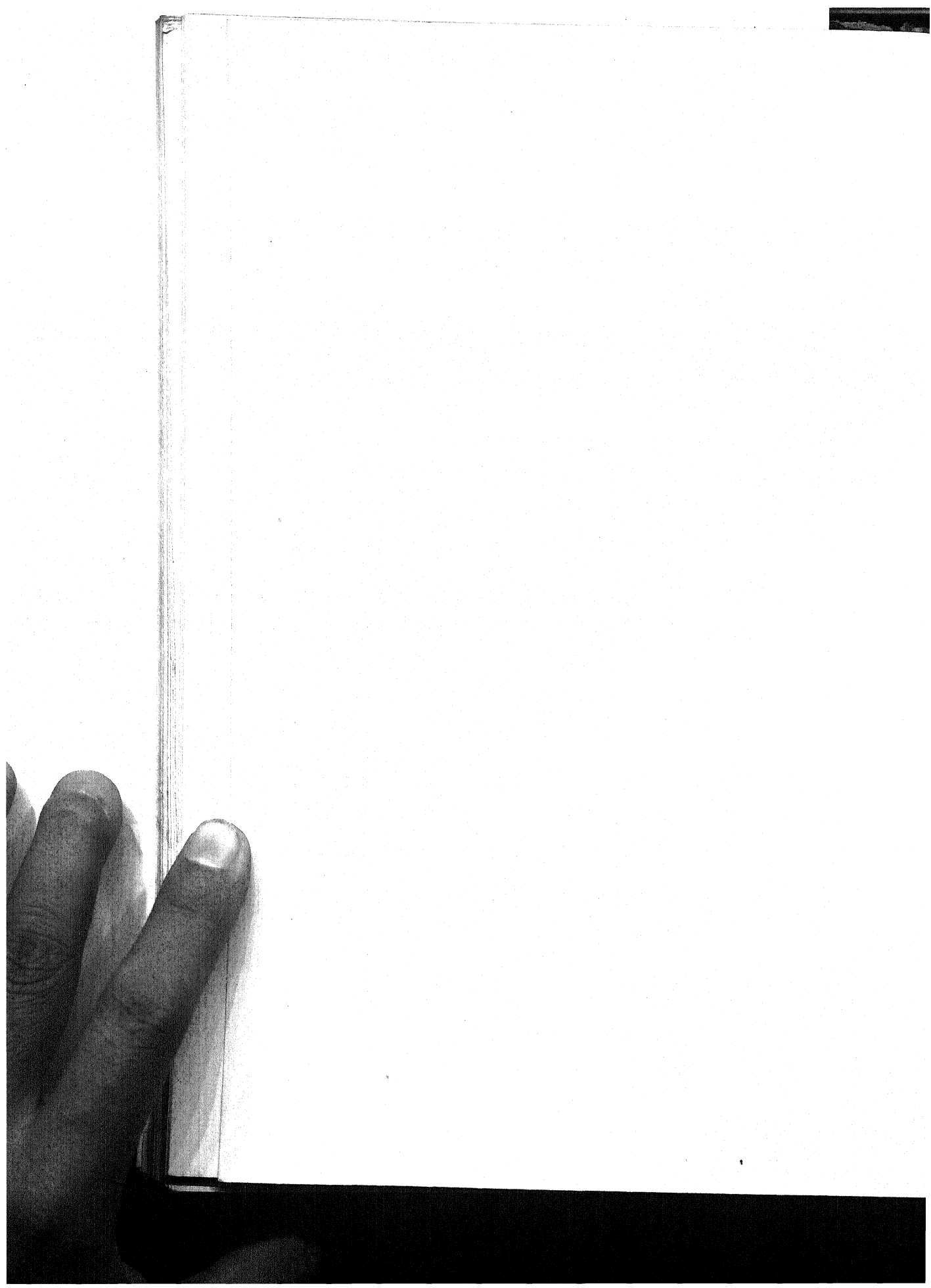
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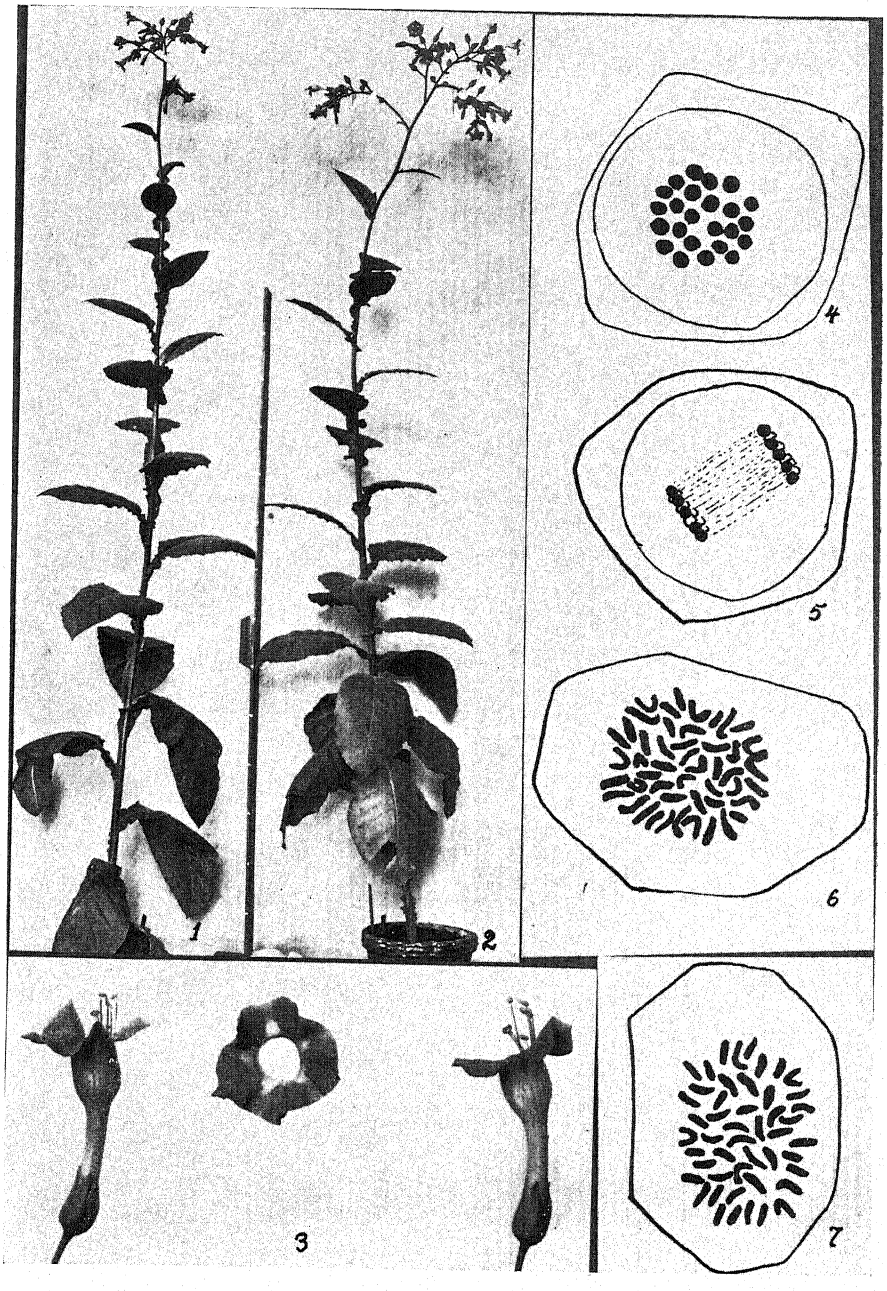
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A FERTILE TRIPLE HYBRID, *NICOTIANA TABACUM* × (*NICOTIANA SYLVESTRIS* × *NICOTIANA RUSBYI*). PRELIMINARY REPORT

DONTCHO KOSTOFF

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The hybrids obtained after crossing *N. Tabacum* ($n = 24$) × *N. sylvestris* ($n = 12$), *N. Tabacum* ($n = 24$) × *N. Rusbyi* ($n = 12$), *N. sylvestris* ($n = 12$) × *N. Rusbyi* ($n = 12$), and from the reciprocal crosses are self-sterile under normal conditions. The cytological studies on these three species hybrids (Kostoff, 1930) showed that in the pollen mother cells (PMC's) of the first two there were 12 bivalent and 12 univalent chromosomes appearing during the first meiotic division, while in the hybrid of the third cross, *N. sylvestris* × *N. Rusbyi*, 24 univalent chromosomes were usually found. The latter cross combination formed occasional dyads with *2n*, i.e. 24, chromosomes instead of tetrads as a result of omission of the first meiotic division. This occurred most frequently when the hybrid plants from this cross grew under conditions of insufficient water supply and intense sunshine. When *N. Tabacum* was pollinated with pollen from the hybrid *N. sylvestris* × *N. Rusbyi* containing a relatively high percentage of pollen grains originating from such dyads, seeds were obtained from which very vigorous plants—triple hybrids, *N. Tabacum* × (*N. sylvestris* × *N. Rusbyi*)—were raised (Pl. X, figs. 1 and 2). Among these triple hybrid plants intermediate types were found as well as those that resembled more closely one of the parental plants in respect to certain characters. The intermediate types (figs. 1, 2, and 3) proved to be fully fertile while the others were only partially so. Cytological studies on both types of plants were made and it was found that the partially fertile plants had a relatively irregular reduction division and usually 48 + 1, or 48 + 2 somatic chromosomes, while the fertile intermediate triple hybrids had normal, regular, reduction division (figs. 4 and 5), and had 24 chromosomes in the PMC's (fig. 4) and 48 in the root tips (figs. 6 and 7). The origin of these fully fertile triple hybrids can be explained on a cytological basis as follows: As manifested by the F_1, half of the chromosomes of *N. Tabacum* ($n = 24$) have an affinity for the chromosomes of *N. sylvestris* ($n = 12$) and the other half (12) have an affinity for those of *N. Rusbyi*, while the chromosomes of *N. sylvestris* do not manifest any such affinity for the chromosomes of *N. Rusbyi* as also observed in the F_1 plants. However, after pollinating *N. Tabacum* with pollen from F_1 (*N. sylvestris* × *N. Rusbyi*) which contained a high percentage of abortive grains and also those originating from dyads with 24 chromosomes (12 from *N. sylvestris* and 12 from *N. Rusbyi* conse-



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quent to the omission of the first meiotic division) the 24 chromosome egg cell of *N. Tabacum* has been fertilized by a generative nucleus with 24 chromosomes so that the resultant triple hybrid has 24 chromosomes from *N. Tabacum*, 12 from *N. sylvestris*, and 12 from *N. Rusbyi*. Since the chromosomes of *N. Rusbyi* ($n = 12$) have affinity for 12 of those of *N. Tabacum* ($n = 24$) and those of *N. sylvestris* ($n = 12$) for the remaining 12 of *Tabacum*, the fertile triple hybrid contains complete haploid sets from all three species and combines the characters of them all, is cytologically balanced, and forms entirely normal gametes. The partial fertility of the other triple hybrids and their greater likeness to one or another of the parent plants in certain characters is apparently due to the excess of 1 or 2 chromosomes that they contain, these chromosomes being apparently contributed by the paternal gametes following non-disjunction during the second meiotic division. Progeny of both the fertile and partially fertile plants are being raised as well as plants from backcrosses with them and *N. Tabacum* and *N. sylvestris;* these will be described elsewhere.

The case reported here appears to be the first instance of a fertile triple hybrid truly composed of complete contributions from the three different species combined.

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DESCRIPTION OF PLATE X

FIGS. 1 and 2. Fertile triple hybrids, *N. Tabacum* × (*N. sylvestris* × *N. Rusbyi*). The ruler between them is marked off into units of 10 cm.

FIG. 3. Flowers from the plants in figures 1 and 2.

FIG. 4. First meiotic metaphase with 24 chromosomes from the plant in figure 2.

FIG. 5. First meiotic anaphase from the plant in figure 2.

FIG. 6. Metaphase with 48 somatic chromosomes in the root tip of the plant in figure 1.

FIG. 7. Metaphase with 48 somatic chromosomes in the root tip of the plant in figure 2.

AN ABERRANT *NICOTIANA* WITH 91 CHROMOSOMES

JAMES KENDALL

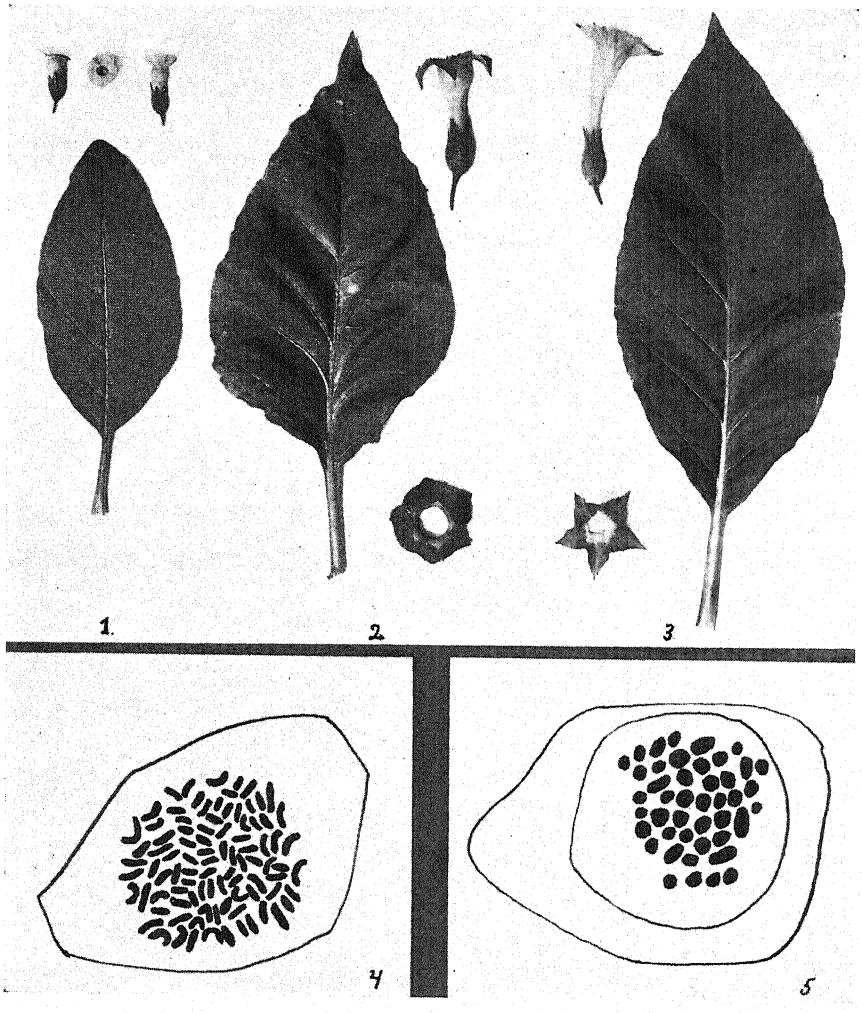
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In a study of certain back-crosses, Kostoff (1930) reported finding among the offspring of F_1 (*N. rustica* × *N. Tabacum*) × *N. Tabacum* one plant with 78 somatic chromosomes and with about 38–40 appearing in the first meiotic metaphases of the pollen mother cells (PMC's). This plant proved to be slightly self-fertile and gave offspring with different chromosome numbers and morphological characters varying greatly from those of the parental plant. Among these plants some were found with 36–38, 40, and about 60 chromosomes in the first metaphases of the PMC's. One plant with 36–38 chromosomes in the first metaphase of the PMC's, irregular meiotic divisions, and viable pollen grains of diverse sizes, when selfed gave seeds from which a number of plants were raised. The most outstanding plant of this progeny apparently arose from the fusion of aberrant gametes for it differed not only in morphological characters from the parent but had 91 chromosomes in its somatic cells.

The morphological characters of the leaf and flower of the aberrant plant are illustrated in Plate XI, figure 2 and the blending of certain of the features of the two species involved in its origin can be easily seen from a comparison with those of *N. rustica* ($n = 24$) in figure 1 and those of *N. Tabacum* ($n = 24$) in figure 3. The flower is much shorter and heavier than that of *Tabacum*, the calyx is much larger relative to the size of the flower, the corolla tube is shorter and in contour resembles that of *rustica* more than *Tabacum;* the petals are a slightly darker red than those of *Tabacum*, much more broadly fused and rounded, and curling backwards when open. The characters of *rustica*—the very small flower yellowish green in color, the broadly fused rounded petals flexed backwards, etc. (fig. 1)—appear to have markedly influenced the moulding of the flower type in this aberrant plant.

The leaf, as shown by figure 2, is larger in every way than that of *rustica* and much broader though somewhat shorter than that of *Tabacum*. Like the flower it is quite distinctly different from either parental type though closer to *Tabacum*. It is much wrinkled and has uneven, wavy margins.

A cytological study of the floral buds at the time of meiosis showed this process to be accompanied by marked irregularities. In the first metaphases of the PMC's 44 chromosomes were usually counted (fig. 5) though other plates were also found where from 40 to 45 chromosomes could be counted during this phase. Apparently, polivalency occurs in this case, a not uncommon occurrence in *Nicotiana* (Kostoff, 1930). In the early anaphase



KENDALL: NICOTIANA

of the first division one or more chromosomes usually led the advance to the poles while in the late anaphase many were often observed lagging along the spindle. In some instances a complete first division apparently failed to take place for only a single metaphase plate with about 80 chromosomes appeared in the second meiotic division. The second division was as irregular as the first and as a result of the irregularities in the two divisions occasional dyads and triads, many unequal tetrads, and some pentads, hexads, etc. were formed leading to a high percentage of abortive pollen grains (c. 60–80 percent) and viable grains of various sizes.

In the somatic cells of the root tips 91 chromosomes (fig. 4) were counted in the metaphase plates. Irregularities were sometimes observed in the mitotic process, one or two chromosomes leading the advance to the poles or lagging on the spindle, and a resultant unequal distribution of these chromosomes accounts for the plates that were occasionally observed with one or two chromosomes more or less than 91.

This aberrant plant proved to be slightly self-fertile and its capsules also became swollen when pollinated with *N. Langsdorffii* ($n = 9$) pollen. Whether the seeds from the latter cross are parthenocarpic or are parthenogenetic remains to be seen.

The author is indebted to Dr. Kostoff for the material for this study.

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DESCRIPTION OF PLATE XI

FIG. 1. Leaf and flowers from *N. rustica*, one of the species used in obtaining the aberrant plant.

FIG. 2. Leaf and flower from the aberrant plant of F_1 (*N. rustica* × *N. Tabacum*) × *N. Tabacum* with 91 chromosomes.

FIG. 3. Leaf and flower from *N. Tabacum* used in the crosses producing the plant of figure 2.

FIG. 4. Somatic metaphase with 91 chromosomes from the root tip of the aberrant plant.

FIG. 5. First metaphase in the PMC with 44 chromosomes from the aberrant plant.

THE LIFE HISTORY OF *PHYSARUM POLYCEPHALUM*

FRANK L. HOWARD

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Of the five hundred or more papers which have been published on the Myxomycetes, the great majority are concerned with descriptions of the mature fructifications and the taxonomy of the group; only a few deal with life histories, although the latter are essential as a basis for sound taxonomy. As yet a complete and detailed account of the development from spore to spore of a single species has apparently never been presented. The following account of the life history of *Physarum polycephalum* is not entirely complete, but is offered with the hope that it may contribute something to our knowledge of a relatively obscure group of organisms.

MATERIAL AND TECHNIQUE

The material upon which this paper is based was first collected as a greenish yellow plasmodium on pilei of *Pleurotus sapidus*. This material was cultured on the pilei of various agarics and upon fructification proved to be *P. polycephalum* Schw. Several gatherings of the plasmodium and of mature sporangia have subsequently been made. Morphological changes from the time of the germination of the spore to the young plasmodium were studied in the following ways: in hanging drop cultures in Van Tieghem cells; in water drop cultures on the surface of slides; and from killed and fixed preparations. Vital stains were also employed to study spore germination and the stages which follow. Skupienski (27) studying *Didymium nigripes* used neutral red, methylene blue, and Janus green B as vital stains in concentrations of 1:5000. In my experiments the concentration was reduced to 1:20,000. Spores were sown in drops of the dye solutions on slides; similar sowings were made in water on check slides, and observations were made at intervals. The myxomycete went through its normal course of development in this concentration of the dyes and abundant young plasmodia were present seventy-two hours after the spores were sown. Neutral red stains the vacuoles, bringing out their size, distribution, and movement clearly. Methylene blue stains the cytoplasm brilliantly. The Janus green B stains both the cytoplasm and the mitochondria. Autoclaved distilled or tap water subsequently aerated by shaking was not deleterious to germination or development and gave an initially sterile medium. It was soon found that water cultures, at best, quickly became contaminated with bacteria and sometimes with protozoa.

The nuclear behavior of the stages from germination to the microscopic

plasmodium was studied from permanent mounts stained either with crystal violet according to the method of Cotner (6) or with Heidenhain's iron-alum-haematoxylin. Allen's modification P. F. A. 3 of Bouin's solution (22) has given the best fixation of all stages in the life history of this myxomycete. The anatomy and cytology of the macroscopic plasmodium has been studied from both smears and paraffin sections stained with iron-alum-haematoxylin. The stages of the development of the sporangia have been studied from paraffin sections.

The Spore

The spores are globose and vary from 8 to 11 μ in diameter. In mass, as seen with the naked eye or hand lens, they are reddish to purplish brown, corresponding to Ridgway's (25) light seal brown or bone brown. Under the higher powers of the microscope the individual spore is brownish yellow in color. The entire surface of the spore wall is minutely spinulose (Pl. XII, fig. 1). Only uninucleate spores have been observed in stained preparations. The nucleus is rather large and has but a single nucleolus. Granules, which stain deeply with iron-alum-haematoxylin and which correspond to the mitochondria described by Cowdry (7), are usually present in the cytoplasm (fig. 2). De Bary (2) and Gilbert (11) record an irregular, highly refractive body in the spore protoplast which is absorbed by the healthy swarmcell before, during, or just after germination. This has been noted in some spores of *P. polycephalum* just after their formation but seems to be absent in spores at germination.

The constancy of the length of the incubation period for a given collection of spores of *P. polycephalum* has apparently been shown by the germination under similar conditions of five lots of spores obtained from sporangia formed in the laboratory. By incubation period is meant the time elapsing between the sowing of the spores and the splitting of the spore walls. Lot 1 uniformly required an incubation period of over forty-eight hours when fresh and when seventeen months old. Lot 2 required about twenty-four hours when fresh and when five months old. Lots 3, 4, and 5 required fifteen to eighteen hours when fresh and four months later the same time was necessary.

The period of activity for the protoplast of *P. polycephalum* begins just prior to the splitting open of the thick yellow spinulose spore wall. In living cultures the protoplast is observed to emerge in one of two ways. In some cases a single large hyaline non-flagellate protoplasmic body emerges from the spore case and then becomes quiescent at the mouth of the crack. In other instances two quiescent hyaline non-flagellate cells, each having about one-half the mass of the one just referred to, can be seen through the crack in the spore wall (figs. 4 and 5). Smith (28) lists thirty-nine species whose spores have given rise to more than one swarmcell upon germination. This phenomenon is apparently general throughout the myxomycetes.

The spore nucleus of *P. polycephalum* always divides once at germination. The nucleus undergoes a typical mitotic division. This division may occur

just previous to the splitting of the spore wall (fig. 3), or when the spore case splits open and before the protoplast emerges (figs. 6–10), or just after the protoplast emerges and becomes quiescent at the mouth of the crack in the spore wall. The thickness of the protoplast and the minute size and clumping of the chromosomes permit no more than a rough estimate of their number. Usually at late telophase a cleavage furrow begins to cut in from the perimeter of the elongate protoplast to separate the daughter nuclei and their contingent cytoplasm into two cells (fig. 13). Division of the protoplast within the split spore case followed by the emergence of two daughter protoplasts is seen most frequently. The number of cells emerging from the spore in this species is merely a matter of the time and place of division. Here, at the beginning of the life cycle, we get a doubling of the number of individuals.

The Swarmcell or Gamete

The first amoeboid daughter cell following emergence becomes quiescent at the mouth of the split in the spore wall. Its protoplasm exhibits a slow streaming which continues for five minutes to four hours. Then the rate of streaming increases and suddenly a uniflagellate amoeboid swarmcell is formed by a change in shape and the putting out of a flagellum. The first daughter swarmcell moves away from the opening in the spore case and the second one usually emerges shortly afterward and soon puts out a flagellum. The direct emergence of a flagellate amoeboid swarmcell from the spore has not been seen. Each flagellated swarmcell (figs. 14, 15) moves about in amoeboid fashion a few minutes by posterior pseudopodia and uses the anterior flagellum as an antenna. Sometimes the amoeboid form retracts its flagellum five to fifteen minutes after emergence and becomes quiescent. Generally, however, the amoeboid movement continues two to thirty minutes when the swarmcell jerks violently once or twice and begins a slow rotating movement. As the rate of the flagellum movement increases, the swarmcell leaves the slide surface, assumes a comma shape (figs. 16–18), and moves rapidly through the upper layers of water in a rotating fashion No feeding takes place during this movement. At times this stage is seen almost to the exclusion of all others. The rotating movement, interspersed by short periods of amoeboid movement, lasts for some time, since the majority of swarmcells exhibit it during the first twenty-four hours, after which it gradually disappears in the cultures.

In stained preparations of the comma-shaped rotating swarmcell we find a beaked nucleus connected by deeply stained threads with the blepharoplast and flagellum (figs. 16 and 17). These connecting threads are termed Geisselglocke by Jahn (17) and rhizoplasts by Wilson and Cadman (29) and Sharp (26). The swarmcell is described as dividing while in a quiescent state, its nucleus dividing mitotically, but thousands of quiescent swarmcells were examined without a clear-cut mitotic figure or dividing cell being found. Clusters of quiescent swarmcells are common in cultures. Cienkowski (5)

applied the term microcyst to these hyaline, non-flagellate, globose cells (figs. 22 and 23). They have a definite but fragile cyst membrane. Continued exposure of the swarmcells of *P. polycephalum* to intense illumination causes them to become quiescent and to form microcysts. Likewise, as moisture becomes limited, the swarmcells retract their flagella and become microcysts. If the intense illumination is removed or if water is added to the culture, a swarmcell is seen to emerge from the microcyst in from one to six hours. The emergence from the cyst membrane takes place in the following manner. A protuberance forms on the side of the globose microcyst and gradually enlarges (figs. 24–28). This process might even be mistaken for division of the microcyst by budding. The knob continues to enlarge until the whole is dumbbell-shaped. Then the protoplast can be seen pulling away from the cyst membrane and in about five minutes the amoeboid cell is entirely free. It remains quiescent about ten minutes and then becomes a flagellated amoeboid swarmcell. In about five minutes the amoeboid form becomes transformed into the rotating form.

Gilbert (9) has pointed out that biflagellate swarmcells of two types are recorded in the literature. One type is characterized by two flagella at the anterior end and the second by one flagellum at each end. The presence of the monopolar biflagellate type of swarmcell in cultures of *Trichia varia* (1), *Stemonitis fusca* (9) and *Dictydiaethalium plumbeum* (10, 28) seems to prove conclusively that the first type does exist. In *P. polycephalum* the occurrence of swarmcells with two anterior flagella is not rare (fig. 20). One flagellum is short and the other very long. Swarmcells with a flagellum at each end were concluded to be protozoa of the genus *Cercomonas* by Gilbert (9). His conclusions have been in part corroborated by the finding of a biflagellate protozoan associated with biflagellate and uniflagellate comma-shaped myxomycete swarmcells in a few cultures of *P. polycephalum*. In these cultures the spores were sown in a drop of autoclaved tap water on well flamed slides. Before germination, however, the original drop evaporated and unsterilized distilled water was added. Besides the typical comma-shaped rotating myxomycete swarmcells (figs. 16–18), a smaller more slender organism with a flagellum extending from each pole (fig. 21) and having a gliding movement was present in considerable numbers. The myxomycete zygote, whose formation by the posterior fusion of two uniflagellate swarmcells is described later, might be confused with a bipolar protozoan but its larger size and characteristic movement readily distinguish it from the latter (compare figs. 21 and 29).

The Zygote

The posterior attachment of two rotating swarmcells followed by plasmogamy to form a zygote has been observed many times in cultures of *P. polycephalum*. The comma-shaped, rotating swarmcell and the gamete are apparently synonymous. By observation of living cultures it was found that

the swarmcells may fuse as gametes without further division occurring aside from the single one when the spore case splits open. Two isogametes were observed to become attached posteriorly (Pl. XII, fig. 29) and to form a zygote two hours after the first spore had germinated and before a single quiescent swarmcell could be found in the culture. Copulation was most abundant, however, six to twenty-four hours after germination began. An explanation of why all the gametes do not pair off and fuse at once has not been satisfactorily formulated. Cayley (4) suggests that in *Didymium* several divisions must occur before the gametes are sexually mature and ready to fuse but this does not seem to be the case in *P. polycephalum*. Wilson and Cadman (29) believe that special nutrient conditions in the germination drop are necessary to obtain numerous sexual fusions of the gametes of *Reticularia lycoperdon*. Fusion of the gametes of *P. polycephalum* takes place in tap or distilled water in abundance without the addition of nutrients.

Only the fusion in pairs of the comma-shaped rotating flagellate type of swarmcell has been observed. Never have amoeboid swarmcells been observed to fuse in pairs as recorded by Jahn (18). The addition of *P. polycephalum* to *R. lycoperdon* (29) and *Didymium difforme* (4), in which motile flagellate gametes fuse to form the zygote, suggests that this type of behavior may be common among the Myxomycetes. The following typical fusion process was observed in a tap water culture eighteen hours after the spores were sown. Two isogametes circled about each other several times, touched at their posterior ends, and separated repeatedly. Then a fine, thread-like pseudopodium was seen stretching between the two as they pulled apart. It finally broke. They again came in contact and a broader pseudopodium was seen between them as they pulled apart. This was stronger and held them together. The attachment gradually became broader and a biflagellate zygote resulted. The anterior flagellum of each gamete continued to move independently which caused the fused gametes (swarmcells) to move about in the water. The fused gametes assumed various shapes (figs. 30–32), each having a characteristic movement. At intervals they settled on the surface of the slide and moved about in an amoeboid fashion with constant change in form (figs. 33–35). After sixty-seven minutes the fused gametes finally settled to the slide surface and eight minutes later the flagella were retracted and the gametes became an amoeba-like, non-flagellate zygote which ingested bacteria. In some cases the zygote instead of continuing its amoeboid movement became a binucleate microcyst (figs. 36, 37) possibly because of intense illumination of the microscopic field. After nuclear fusion and subsequent division, the zygote, it is believed, becomes the young plasmodium (figs. 38–43) although a complete series of steps showing the origin of the plasmodium from the zygote is lacking. The permanent multiple fusion of swarmcells to form a young plasmodium was never seen.

The Plasmodium

Food Relations

Plasmodia have usually been observed and described in their native habitat. A. Lister (19) cultivated the plasmodium of *Badhamia utricularis* on various Hymenomycetes. Since then several other mycophagous species have been found and cultivated in the laboratory. Macbride (21) found a yellow plasmodium spreading over a fruit-body of *Agaricus* (*Pleurotus*) *sapidus*. He brought it into the laboratory, placed it under a large bell jar, and fed it more of the same agaric. Typical sporangia of *P. polycephalum* Schw. were formed from a portion allowed to fruit. Currie (8) likewise cultivated the yellow plasmodium of *P. polycephalum* in the laboratory.

In my experiments the plasmodium of *P. polycephalum* has been kept in culture for about eighteen months. At first the plasmodium was fed fleshy agarics, and then various Polyporaceae and Thelephoraceae during the months when agarics were not available. Finally, pure cultures of fungi growing on nutrient agar were used as food. The rapidity with which the spores, lamellae, and trama of a fresh agaric are utilized may be seen in Plate XIV, figure 46, which shows what remains of an agaric twenty-four hours after a small piece of plasmodium had been transferred to its surface. The fructifications of various Polyporaceae and Thelephoraceae serve as a fair substratum for growth of the plasmodium. The plasmodium creeps over their hymenial surface, digesting and absorbing the tenderer structures (figs. 47, 48). It is necessary to change them but once or twice a week, by which time they become badly infested with mucors and bacteria.

The use of sterilized fungus fruit-bodies as a medium on which to grow the plasmodium in pure culture was suggested upon observing that the plasmodium both maintained itself and grew on mushrooms. A decoction composed of macerated mushrooms, water, and one and one-half percent agar, was prepared and sterilized. Fragments of contaminated plasmodium as small as one square centimeter were transferred to this medium in petri plates. These pieces lived and enlarged on the mushroom agar (Pl. XV, fig. 51). The piece of plasmodium must be free of bacteria when transferred, or the medium must be made slightly acid, otherwise bacterial growth will quickly become excessive and kill the plasmodium. In contaminated plates it has repeatedly been observed that the plasmodium moves away from the bacterial colonies, which might be an indication that the latter are not utilized as food. Plasmodia can be freed of bacterial contamination by repeated transfer on acid media. Uncontaminated plasmodia were transferred to plugs of sterilized *Polyporus frondosus* in test tubes. They grew rapidly and in about three days increased in bulk many times. Sometimes a portion of the plasmodium after several days feeding moved to the upper, drier part of the tube and formed a bright orange sclerotium while the remainder continued to feed. Sporangia have not been formed from plasmodia in pure culture in petri plates or test tubes where pieces of sterilized mushroom were used for food.

Attempts were next made to grow the plasmodium on various kinds of nutrient agars. Knop's synthetic agar (24) alone and supplemented with two percent sucrose or two percent maltose were tried. Fragments of plasmodium were maintained in an active condition for thirty days on these media without increase in mass. The acidity of the medium checked bacterial growth. Similarly, dung decoction, prune and various nutrient agars did not provide the nutrients necessary for growth but merely for maintenance of the plasmodium. Acidified oat meal agar is the one medium of those tried which gives promise of being utilizable by the plasmodium. Small fragments of plasmodium contaminated with a toruloid yeast (an imperfect budding form) when transferred to this medium thrived and increased their mass. The role the yeast played in the nutrition of the plasmodium was not ascertained, since yeast-free plasmodia were not available to check this point.

In conjunction with the above culture experiments the use of pure cultures of fungi as food was tried. This has furnished a method for growing plasmodia in quantity as desired. A piece of bacteria-free *P. polycephalum* plasmodium ($\frac{1}{2} \times 1$ cm.) was transferred to a flask of soft Knop's agar plus two percent sucrose upon whose surface an *Alternaria* colony was growing. The plasmodium moved to the fungus colony and within twenty-four hours the mycelium was assimilated. The plasmodium doubled in volume and moved on. The large muriformly septate *Alternaria* spores were left untouched as a dark green patch. The plasmodial fragment had not increased in size on the petri plates of agar upon which it had been repeatedly transferred while freeing it of bacteria. The *Alternaria* spores germinated and formed a new mycelium within a few days. The plasmodium returned to the mycelium, assimilated it, again doubled in size, and moved on, leaving the fungus spores. This continued until the *Alternaria* completely covered the agar surface with its spores. By this time the plasmodium was very large. It moved up the sides of the flask one night and formed normal sporangia near the cotton plug.

Pure cultures of various fungi in large flasks were then tested as food for the plasmodium of *P. polycephalum*. Various species of Mucorales and *Guepinia spathularia* are untouched by the plasmodium. The spores of *Penicillium* sp. and *Alternaria* sp. are untouched but the hyphae are utilized. The hyphae of *Merulius americanus* are utilized fairly well but some residue is left. The hyphae of *Lenzites betulinus*, *Tremella* sp., *Tremella mesenterica*, *Exidia glandulosa*, *Nidularia pulvinata*, and *Cyathus stercoreus* are utilized completely. Sporangia were formed in all of the above cultures where the fungus was capable of being used for food.

A plasmodium about one centimeter square, when transferred to a flask containing mycelium of *Tremella mesenterica*, increased in area to about twenty-five square centimeters within seven days. Then it formed sporangia normal in size, shape, etc. except that the peridium was beautifully

iridescent as in *Diachaea*. Explanation of the iridescent peridium was forthcoming by observation of the development of sporangia on four successive occasions from a plasmodium feeding on *Lenzites betulinus* hyphae (fig. 49). The first two times the plasmodium moved up the moist walls of the flask near the cotton plug and formed sporangia with an iridescent peridium. Each time a small portion of the assimilative plasmodium refrained from developing into sporangia. This returned to the agar and continued to feed on the *Lenzites* hyphae and to increase in size. In the meantime, the culture flask was becoming drier and by the time the plasmodium had attained bulk enough to form sporangia a third time, the flask walls were not covered with condensed moisture. This time the plasmodium moved only a short distance up the sides of the flask and normal sporangia with a gray lime-covered peridium were formed. A small portion of the plasmodium again returned to feed and grow on the fungus hyphae. The fourth time the entire plasmodium developed into normal, erect calcareous sporangia directly on the surface of the rather dry agar. Excessive humidity, therefore, seems to prevent the concentration of lime on the peridium.

Morphology and Cytology

A containing layer around the plasmodium may easily be demonstrated by cutting one of the strands transversely with a razor blade. The semi-fluid, light yellow contents flow out and form a large rounded drop. The rapidity with which the contents are forced out suggests that they are under pressure. Sometimes the strand re-connects itself but many times the two severed portions remain separate. Stained paraffin sections of a plasmodial strand show a definite peripheral layer, seemingly of protoplasm in a gel-like state. The protoplasm has a vesicular or fibrillar structure in the center of the strand, and is more homogeneous at the periphery (figs. 53, 54). The relative amounts of the two types of protoplasmic structure seem to vary with the stage and rapidity of protoplasmic streaming in the particular strand at the time of its fixation. Only resting nuclei have been seen in sections of these strands. The nuclei are 2.5 to 3.5 μ in diameter and globose to ellipsoidal (fig. 44). They contain either one large or two or three smaller nucleoli and are surrounded by a layer of cytoplasm and a definite nuclear membrane.

The mode of nuclear division in the macroscopic plasmodium of a member of the Endosporeae is still in need of elucidation. Up to the present time nuclear division by mitosis in the plasmodium has been reported in one species only, *Badhamia utricularis*, and from preparations made by J. J. Lister (20) and Jahn (18). Infrequent observation of mitotic nuclear division is remarkable when one considers the numerous attempts made by many workers to find it. Plasmodia of *P. polycephalum* have been grown from small fragments to areas covering two square feet. Relatively the same number of nuclei was present per unit volume in the large plasmodium

as in the transferred fragment. Therefore, it is certain that frequent nuclear divisions must occur.

To determine the mode of nuclear division in *P. polycephalum* series of smear preparations were made. Four trials, using actively growing plasmodia, were made. In the first two trials, samples of the two plasmodia were taken every half hour from 11:30 A. M. to 3 P. M. Another trial was made using hourly samples of a small plasmodium beginning at 11:00 A. M. and continuing until 3:00 P. M. Then, a more extensive trial was made on a large plasmodium from which samples were taken every half hour from 11 A. M. until 12 midnight. A careful scanning of the stained preparations revealed only resting nuclei (fig. 45).

The Sclerotium

The plasmodium of *P. polycephalum* becomes sclerotized when moisture is gradually reduced. Brandza (3) gives a detailed account of the process of dessication of plasmodia and of the morphology of the sclerotia of calcareous Myxomycetes. The color of the sclerotium of *P. polycephalum* varies from a bright orange to a dark brown. It has been reactivated after eight months in the laboratory. The relative humidity of the atmosphere seems to be by far the most important factor influencing sclerotium formation. An abundance of oxygen is necessary in addition to moisture for the reactivation of the sclerotium and the development of the plasmodium.

Development of the Sporangium

The plasmodium of *P. polycephalum* has formed sporangia more than forty times during the course of these experiments. Each time the transformation has occurred only at night. The nocturnal formation of sporangia by Myxomycetes is apparently quite common since it has been described by de Bary (1), Miller (23), Jahn (15, 16), and Lister (19). The mere presence of food does not prevent the plasmodium from forming sporangia. Many times, plasmodia of *P. polycephalum* form sporangia when a great abundance of fresh agaric material is present. It was repeatedly observed that as the substratum became fouled with bacteria the plasmodium would leave it and fruit. If, however, the foul remnants were removed and fresh mushrooms were supplied, the plasmodium continued to feed and increase in size. It was somewhat disturbed in the process and this may have checked fruiting. In my experience a minimum area of about twelve square centimeters must be present before the plasmodium of *P. polycephalum* will develop into sporangia.

In anticipation of fruiting the plasmodium usually assumes a very dense, opaque yellow color; is seemingly gorged with nutrient material and moves rapidly in a broad, fan-like formation to seek a drier location. The advancing margin of this fan sometimes moves forward at the surprisingly rapid rate of almost three centimeters an hour. Upon reaching a suitable situa-

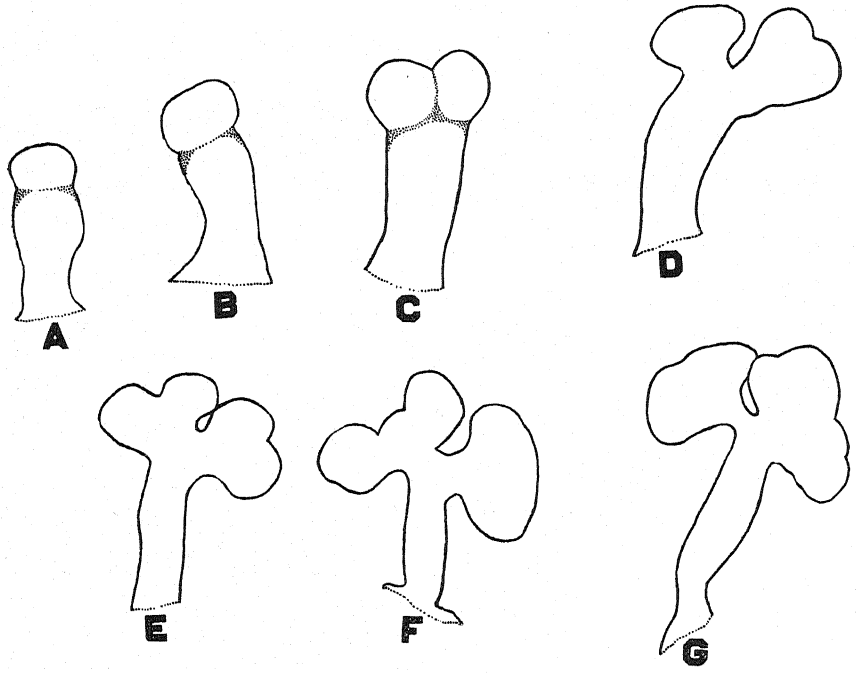
tion, a rather thick, continuous, verrucose layer is formed (fig. 55) by massing of the plasmodium. Cases have occurred, however, in moist flask cultures in which the plasmodium instead of massing formed into strands from which the sporangia arose directly (fig. 50). The more or less continuous layer is apparently split into segments in an indefinite manner by furrows (fig. 56). The furrows begin to form at the upper surface and to cut down through the protoplasm. About one hour elapses before the segments are clearly defined. When segmentation is completed, we find in a tangential section (Pl. XVI, fig. 57) a differentiated homogeneous cortical layer on the surface exposed to the air. At this stage resting nuclei and a few scattered large brown granules of waste material can be found in this layer. The protoplasm of the body of each segment has a highly reticulate structure with nuclei scattered throughout and yellow-brown rounded granules in the vacuoles.

The flat segments round up into knobs during the next hour and the cortical layer shrinks from twenty microns to about ten microns in thickness, becoming slightly denser at its outer edge (fig. 59). This layer is present only on the upper external surface of the knob. A distinct yellow surface membrane is formed over the exposed surfaces of the sporangium initial (fig. 57). It is one to two microns in thickness and follows the contours of the segment. The main body of the protoplasm in the knob retains its reticulate structure and scattered nuclei (fig. 60). As the protoplasm moves upward, the scattered granular material in the vacuoles of the reticulate protoplasm is moved toward the center. Material fixed in corrosive sublimate-acetic-formalin solution shows these brown granules while material fixed in Bouin's solution shows them indistinctly.

The knobs then lengthen out into vertical, finger-like pillars (fig. 58) and the stipe and the sporangium become distinct. The sporangium first appears at the apex as a capitate enlargement of yellowish white protoplasm. A line of demarcation is visible between the stipe and sporangium but at no time does a membrane separate the sporangial head from the stipe. A cross-section at this stage shows an extremely open reticulate protoplasmic structure in the central portion of the stipe where the brownish granules are gathering. The latter are soon aggregated into a rather compact core up which the protoplasm flows into the developing head (fig. 61). During the next hour the sporangial head is rapidly formed. The apical swelling of the finger-like pillar enlarges and divides dichotomously into two lobes. This process of successive dichotomous lobing continues until a large convolute sporangial head is formed (text fig. 1, *A–G*). The sporangium is not compound since all of the lobes are continuous with the central portion although some of them are connected only by very small openings.

The stipe is morphologically complete before the sporangium is completely outlined and capillitium formation takes place. On the outside is a tough, yellow-brown, irregular lamellate wall (fig. 61). Inside of this wall

we find a cylinder of protoplasm having a foam-like structure and a central core of brown granular material which forms the framework and gives rigidity to the stipe. When plasmodia were fed only agarics for a considerable time these granules were few in number in the resulting stipes, which were unable to stand erect under the weight of the sporangia. In the core of the



TEXT FIG. 1, *A* to *G*. Development of *Physarum polycephalum* sporangium by successive dichotomous lobing.

stipe is concentrated the bulk of the yellow pigmentation of the plasmodium. As more and more protoplasm mounts to the apex to form the enlarging sporangial head, the diameter of the stipe becomes less and less until finally nothing but the yellow, twisted, membranous wall and the granular core remain.

It seems as though the protoplasm of the upper lobes changes from a reticulate, open structure to a homogeneous structure before all of the protoplasm from the stipe ascends to the sporangium (Pl. XVII. fig. 62). Wilson and Cadman (29) believe that certain areas of protoplasm in the developing sporangium of *Reticularia* become reticulate in structure and degenerate to form the pseudo-capillitium. In *P. polycephalum* a different interpretation must be put upon the reticulate protoplasm. In examining a cross-section of a developing sporangial head of *P. polycephalum*, areas of reticulate protoplasm may sometimes be found scattered amongst areas of homogeneous

protoplasm due to the plane of section, but if a median longitudinal section is examined, a gradual transition from the reticulate structure to the homogeneous structure may be observed from the attachment of the stipe upward. The lobing of the sporangial head may cause some distortion. Therefore, in this species selected areas of protoplasm do not degenerate to form the capillitium but the latter is formed by secretion into vacuoles essentially as described by Harper and Dodge (14).

The capillitium is developed almost simultaneously with the change to the homogeneous protoplasmic structure (fig. 62). Anastomosing vacuoles are the first evidence of the capillitium. In prepared slides the progressive formation of these lacunae can be seen. They are initially 4.5 to 6.0 μ in diameter but shrink as the threads become mature. Each lacuna in cross-section seems to be bounded by a very thin membrane and to be surrounded by a slightly denser zone of protoplasm. The resting nuclei are not grouped about the lacunae but are scattered in the protoplasm. Fibrillar asters have not been observed around the lacunae at any time during capillitium formation as described for *Trichia* by Harper and Dodge (14). When the capillitium is first formed lime granules are scattered throughout its length as in *Badhamia* but very soon it becomes differentiated into lime-filled nodes and hollow, limeless, thread-like internodes 0.8 to 1.2 μ in diameter. The capillitial threads are attached to the outside peridial membrane and branch freely at the nodes. The capillitium is completely differentiated before nuclear division takes place.

Immediately following capillitium formation and preceding nuclear division, two types of nuclei can be distinguished. One type stains lightly with iron-alum-haematoxylin while the other stains densely (fig. 63). The former are usually larger, measuring 3.2 to 4.8 μ in diameter, and have a granular nucleoplasm. The nuclear membrane and nucleolus are poorly defined. Ten of these are visible to one of the densely stained type. The latter have a clearly visible nuclear membrane, a foam-like nucleoplasm, a deeply stained nucleolus, and measure 2.5 to 3.5 μ in diameter. Harper and Dodge (14) mention two types of nuclei at about this stage in the development of *Hemitrichia clavata*. They suggest that the dense, smaller nuclei are either young daughter nuclei or are degenerating and favor the latter view. They offer no comments on the larger nuclei. I believe the densely stained nuclei are degenerating and suggest that the larger nuclei are undergoing division and are in early prophase. Transitional stages in the degeneration of the densely stained nuclei may be seen in which they become denser and denser until finally they are merely opaque, dark masses. In a given sporangial lobe mitosis is slightly progressive, being more advanced near the periphery and less so toward the interior. Thus early stages of mitosis may be observed near the periphery while resting nuclei are present in the center of a lobe.

Definite structural changes in the nuclei during prophase are very hard to

observe because of their minute size and due to the staining properties of the nucleoplasm. In late prophase the chromatin can be seen taking shape scattered in the nucleoplasm. The nucleolus has entirely disappeared. The chromosomes then gather into a central plate and a spindle is formed. By careful focusing a very delicate membrane may be discerned around some of the dividing nuclei at metaphase (fig. 66).

Cleavage furrows are first observed along the capillitial threads and then pushing in from the periphery of the sporangium. They cut through undifferentiated protoplasm (figs. 64 and 65) and are not preceded by a hyaline or granular zone. Harper (12) gives full details of this progressive process.

Generally the prophase precedes cytokinesis and the later phases of mitosis occur concurrently with cytokinesis. Metaphase must persist for a considerable time for it is most commonly observed in preparations (fig. 66). A very prominently stained centrosome is present when Bouin's solution is used for fixation. The time consumed to complete nuclear division and cytokinesis is about thirty minutes, at most not over sixty minutes. I have been unable to find the nuclear figures positively in pairs suggesting the second division of meiosis as observed by Wilson and Cadman (29). During division the nuclei are not uniformly oriented as Harper (13) found them to be in *Didymium*, for lateral and polar views of mitotic figures may appear side by side. Neither are they all found bordering the cleavage furrows for some are scattered indeterminately throughout the homogeneous cytoplasm.

Mitotic figures, observed when cleavage has progressed, have a very distinct achromatic figure, especially the mantle fibers and centrosomes (fig. 68). Connecting fibers are formed between the separating chromosomes Pl. XVIII, figs. 68 and 69), but no thickening of these has been seen to suggest cell plate formation. At late telophase mitotic figures measuring eight microns from pole to pole have been noted (fig. 70). The clumped chromosomes measure $1.0 \times 2.0\ \mu$. The connecting fibers are about six microns long and very prominent. The two daughter nuclei become crescent-shaped and the connecting fibers disappear. The final cleavage into uninucleate segments is accomplished by furrowing.

Within one-half hour after cytokinesis is completed the segments begin to differentiate themselves into spores. In a section of a normal sporangium the protospores have but a single resting nucleus and nucleolus although rarely a young spore having two nuclei may be seen. Degeneration of superfluous nuclei as found by one of Jahn's students (28) has not been observed in the protospore. Only in sections of an abnormal sporangium, caused by disturbance during development, were multinucleate giant uncleaved protospores seen.

In preparations just after the final cleavage the young spores are polygonal due to mutual pressure (fig. 71). Open furrows are rare or absent. A continuous intersporal membrane separating the young spore protoplasts is formed by secretion into the cleavage furrow. From this the spore wall

is formed. Its existence may be brought out by examining preparations where slow or unequal fixation has occurred. The protoplasts of the young spores near the exterior are unshrunken and the intersporal membrane is more or less hexagonal corresponding to the shape of the cell (Pl. XIX, fig. 72). Toward the interior of the section, the protoplasts are conveniently shrunken making the extended and hexagonal intersporal membrane plainly visible (fig. 73). It is a continuous membrane. Later stages in which the intersporal membrane splits to form the spore wall can be seen in other lobes of the same sporangial section. After splitting, the walls pull apart. During this pulling apart, irregularities are visible on the outer surface which develop into the minute spinules covering the mature spore wall. The spore wall of other species has been described as arising by the secretion of a wall by each young spore about itself. This interpretation of the phenomenon in *P. polycephalum* is therefore unique.

Each spore protoplast contains many deeply stained, round granules in the cytoplasm corresponding to the mitochondria of Cowdry (7) whose nature and derivation are unknown. Also about one in four protoplasts contains a large densely stained mucilaginous body approximately the size of the nucleus (fig. 74). Within twelve hours the transition from a plasmodium to pallid neutral gray to yellowish gray, lobed, mature sporangia filled with spores is completed (fig. 75).

Summary

The life cycle of *Physarum polycephalum* is initiated at the splitting of the wall of the spore after fifteen to seventy-two hours incubation in a drop of water. At about the time of germination the nucleus of the spore always divides once mitotically. This may be just before the spore wall splits, or just after it splits but before the protoplast emerges, or just after the emergence of the protoplast. Whether the number of cells seen to emerge from the spore is one or two depends upon the place and time of this nuclear and subsequent cell division. After a quiescent period following emergence from the spore case each daughter cell puts out an anterior flagellum and becomes first an amoeboid swarmcell and then changes to a comma-shaped rotating one. Intense illumination and moisture conditions may cause the swarmcell to retract its flagellum and to encyst. When the unfavorable condition is removed the protoplast may emerge from the cyst membrane and again become a flagellated swarmcell.

Motile comma-shaped swarmcells function as isogametes which fuse posteriorly in pairs to form zygotes. It is believed that after nuclear fusion and division of the fusion nucleus the plasmodial stage is initiated, although a complete series of steps is lacking. The multiple fusion of swarmcells to form the plasmodium was never observed. The mycophagous extramatrical habit of the plasmodium has facilitated its study. Bacteria when abundant are detrimental to the plasmodium and may bring about its disintegration.

The plasmodium may be slowly dried and the resulting sclerotium later reactivated at any time up to at least eight months by placing it under moist aerobic conditions. The plasmodial strands have a differentiated cortical layer and a highly reticulate protoplasmic structure. In the plasmodium, nuclei have been found only in the resting condition. The plasmodium becomes transformed into sporangia only at night. The transition from a plasmodium to mature sporangia requires about twelve hours under favorable conditions.

The change of the open plasmodium to a continuous verrucose layer, subsequent segmentation of this layer and formation of knobs, followed by vertical pillars and a successively dichotomously lobing sporangial head have been followed in detail. Stipe formation proceeds in a definite manner. The structure of the protoplasm changes from reticulate to homogeneous in the sporangial head. Capillitium formation is by secretion and not by protoplasmic disintegration and it is completed before mitotic division. The capillitium is at first badhamoid and then becomes differentiated into lime-filled nodes and limeless internodes. Nuclei of two types may be distinguished: larger, lightly stained ones which undergo mitotic division, and smaller, densely stained ones which disintegrate. Cytokinesis occurs concurrently with the mitotic nuclear division preceding spore formation. Apparently only a single nuclear division requiring about thirty minutes for completion takes place. Paired nuclei suggesting a second division have not been observed. Following final cleavage of the protoplasm into uniformly hexagonal, uninucleate masses, a continuous intersporal membrane is formed in the furrows. This splits longitudinally to form the spore walls. After splitting, the walls pull apart and the spores become spherical. During this pulling apart, irregularities become visible on the adjoining surfaces which develop into the minute spinules on the mature spore wall.

This study was carried on in the Mycological Laboratory of the State University of Iowa under the direction of Dr. G. W. Martin to whom sincere acknowledgment is given for his counsel and inspiration during the course of the investigation and for his help in the preparation of the manuscript.

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DESCRIPTION OF PLATES

The drawings were made with the aid of a camera lucida and are magnified approximately × 1600. Figures 24 to 28 and figures 31, 32, 34, 35 and 36 were drawn from living material.

PLATE XII

FIG. 1. External view of spore showing spinulose spore wall.
FIG. 2. Optical section of spore showing nucleus and mitochondria.
FIG. 3. Mitotic nuclear division (anaphase) in unopened spore case.
FIG. 4. Emergence of amoeboid daughter cell.
FIG. 5. Quiescent daughter cells in split spore case.

FIG. 6. Single large vacuolate protoplast with resting nucleus in split spore case.

FIGS. 7–9. Stages of nuclear division.

FIG. 10. Two daughter nuclei in uncleaved spore protoplast.

FIG. 11. Emergence of spore contents while the nucleus is in telophase.

FIG. 12. Nucleus dividing after partial emergence of protoplast from spore case.

FIG. 13. Cleavage of binucleate protoplast into two daughter cells.

FIGS. 14 and 15. Amoeboid swarmcells.

FIGS. 16–18. Comma-shaped rotating swarmcells. A prominent blepharoplast and rhizoplast apparatus are present. The nucleus in figure 18 has two nucleoli.

FIG. 19. Rotating swarmcell with an unusually long flagellum.

FIG. 20. Rotating swarmcell with two flagella.

FIG. 21. Biflagellate protozoan which has a gliding movement.

FIGS. 22 and 23. Hyaline microcysts. The protoplast in figure 23 is shrunken showing the cyst membrane.

FIGS. 24–28. Stages in the emergence of a swarmcell from a microcyst.

PLATE XIII

FIG. 29. Posterior fusion of two isogametes.

FIG. 30. Swimming form of zygote which rotates irregularly due to the flagella moving independently.

FIG. 31. Rotating plate-like form of zygote.

FIG. 32. Rotating form of zygote which swims forward in a twisting fashion.

FIGS. 33 and 34. Amoeboid forms of the flagellated zygote.

FIG. 35. Final amoeboid form of zygote before retraction of the flagella.

FIG. 36. Quiescent zygote preceding encystment due to intense illumination.

FIG. 37. Encysted zygote showing cyst membrane where the protoplast has shrunken in fixation.

FIGS. 38–43. Amoeboid zygote. This series shows the binucleate zygote after retraction of the flagella, the nuclei fusing, a probable fusion nucleus with two nucleoli, a resting fusion nucleus, the fusion nucleus dividing, and the young plasmodium with two nuclei.

FIG. 44. Types of resting nuclei found in plasmodial smears.

FIG. 45. Portion of a plasmodial smear preparation showing resting nuclei and cytoplasmic granules.

PLATE XIV

FIG. 46. Agaric upon which a piece of plasmodium has fed for twenty-four hours. × ½.

FIG. 47. Plasmodium digesting hymenium of a polypore. × 2.

FIG. 48. Plasmodium digesting hymenium of a polypore. × 12.

FIG. 49. Plasmodium mounting walls of flask after feeding on fungus hyphae growing on agar. Note sporangia which have formed previously near the mouth of the flask. × ⅓.

FIG. 50. Sporangia arising directly from plasmodial strand in a flask culture. × ¾.

PLATE XV

FIG. 51. Plasmodial fan on agaric agar. × ½.

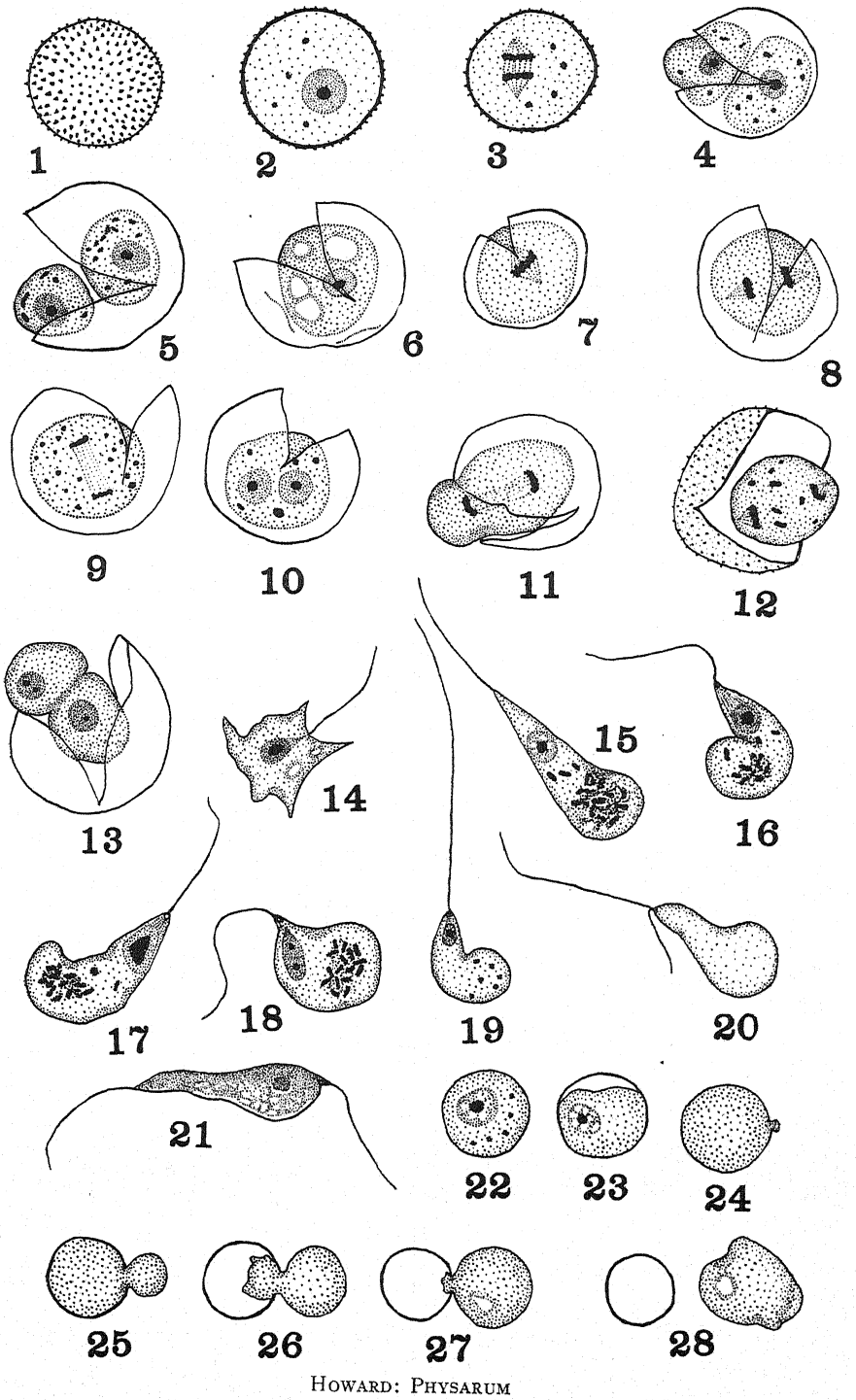
FIG. 52. Plasmodium feeding on *Alternaria* hyphae in pure culture. × ½.

FIG. 53. Transverse section of plasmodial strand, fixed while quiescent. Note cortical layer. × 150.

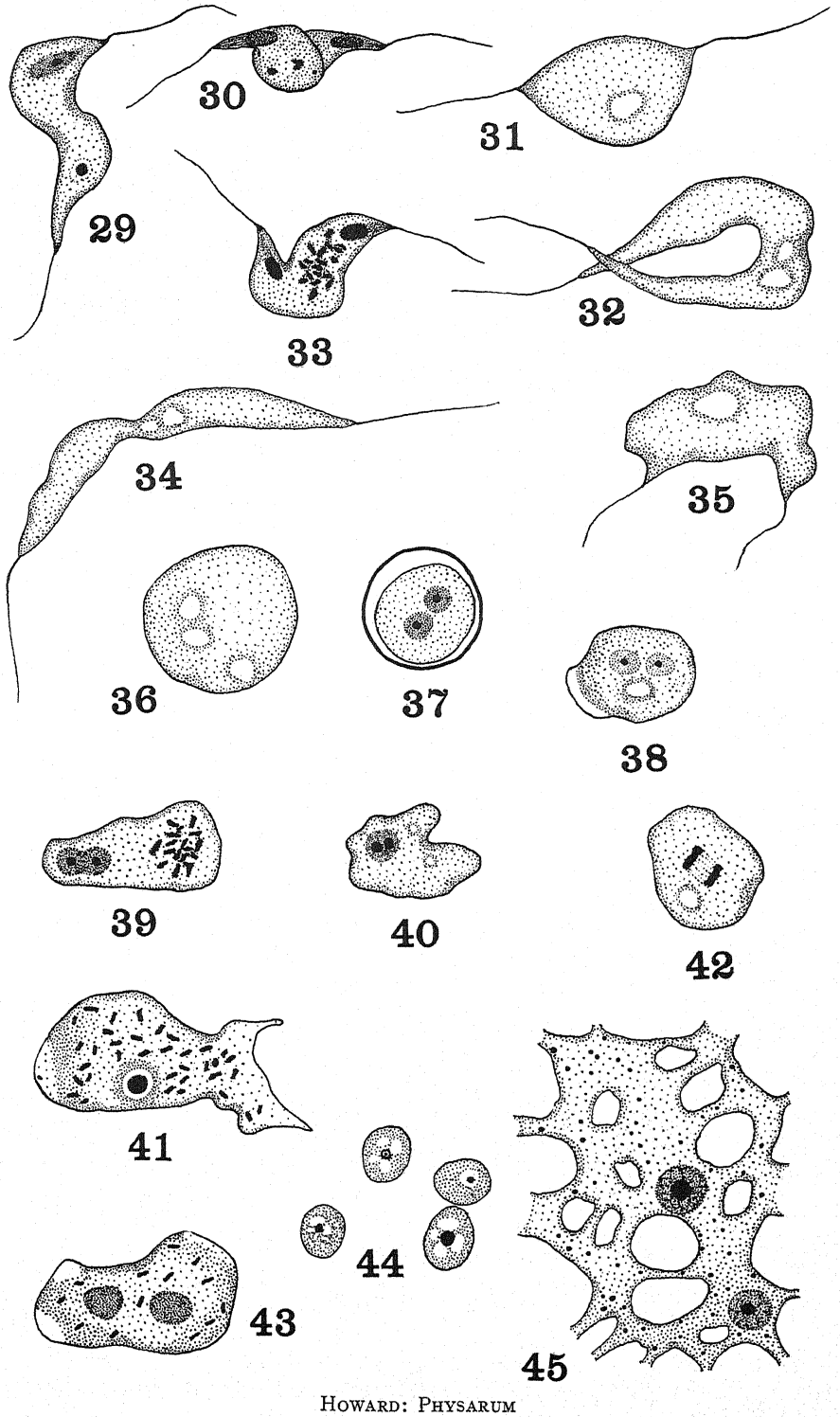
FIG. 54. Transverse section of plasmodial strand, fixed while streaming. × 150.

FIG. 55. Massed verrucose layer of plasmodium; first stage of sporangial development. × 5.

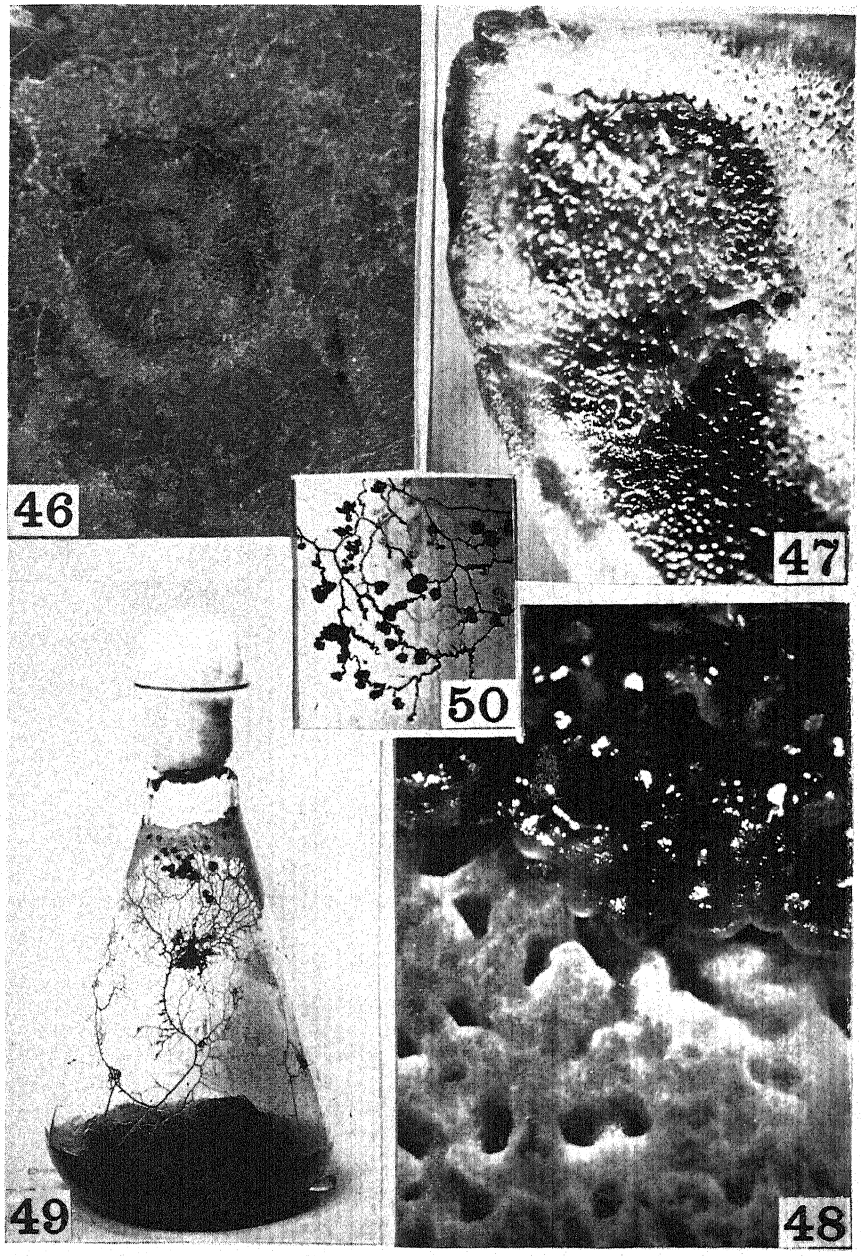
FIG. 56. Segmentation of the verrucose layer; second stage of sporangial development. × 5.



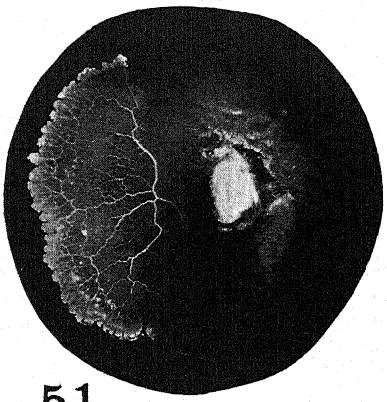
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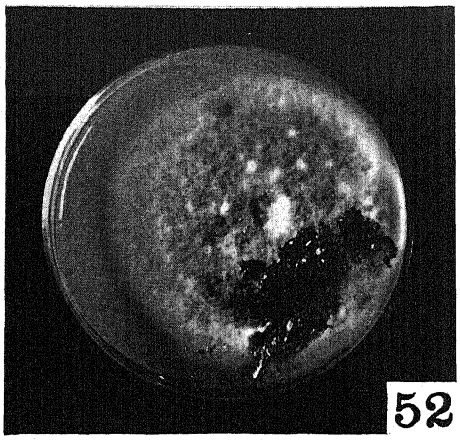
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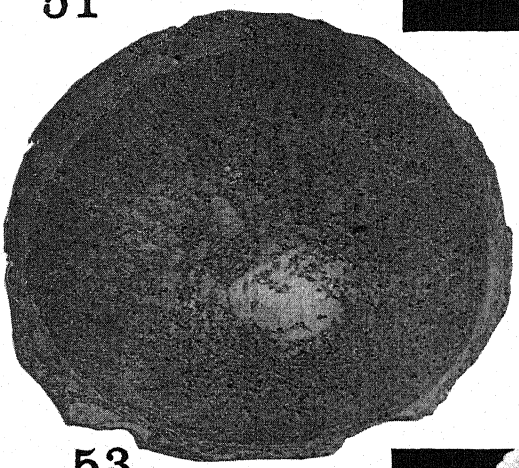
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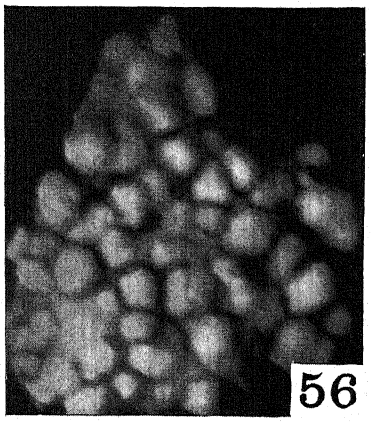
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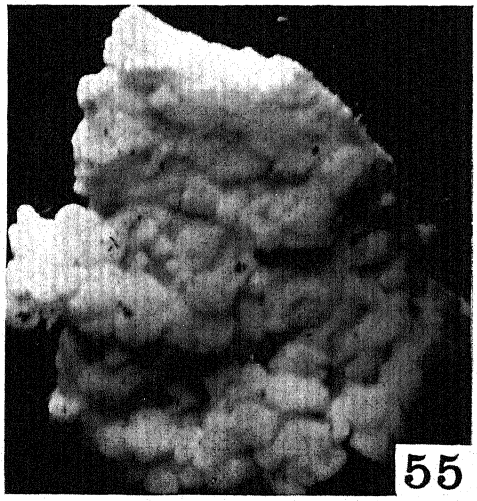
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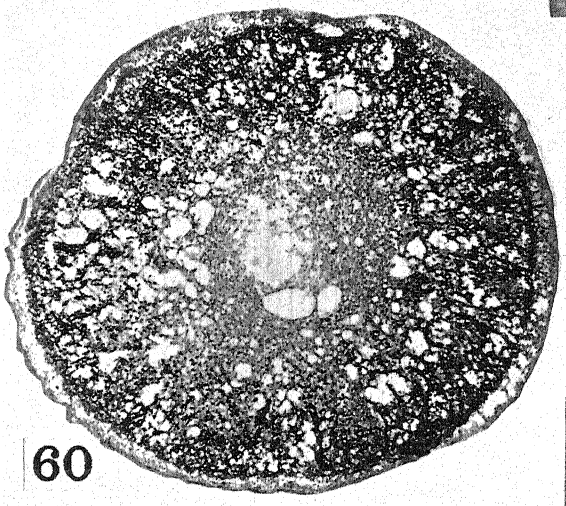


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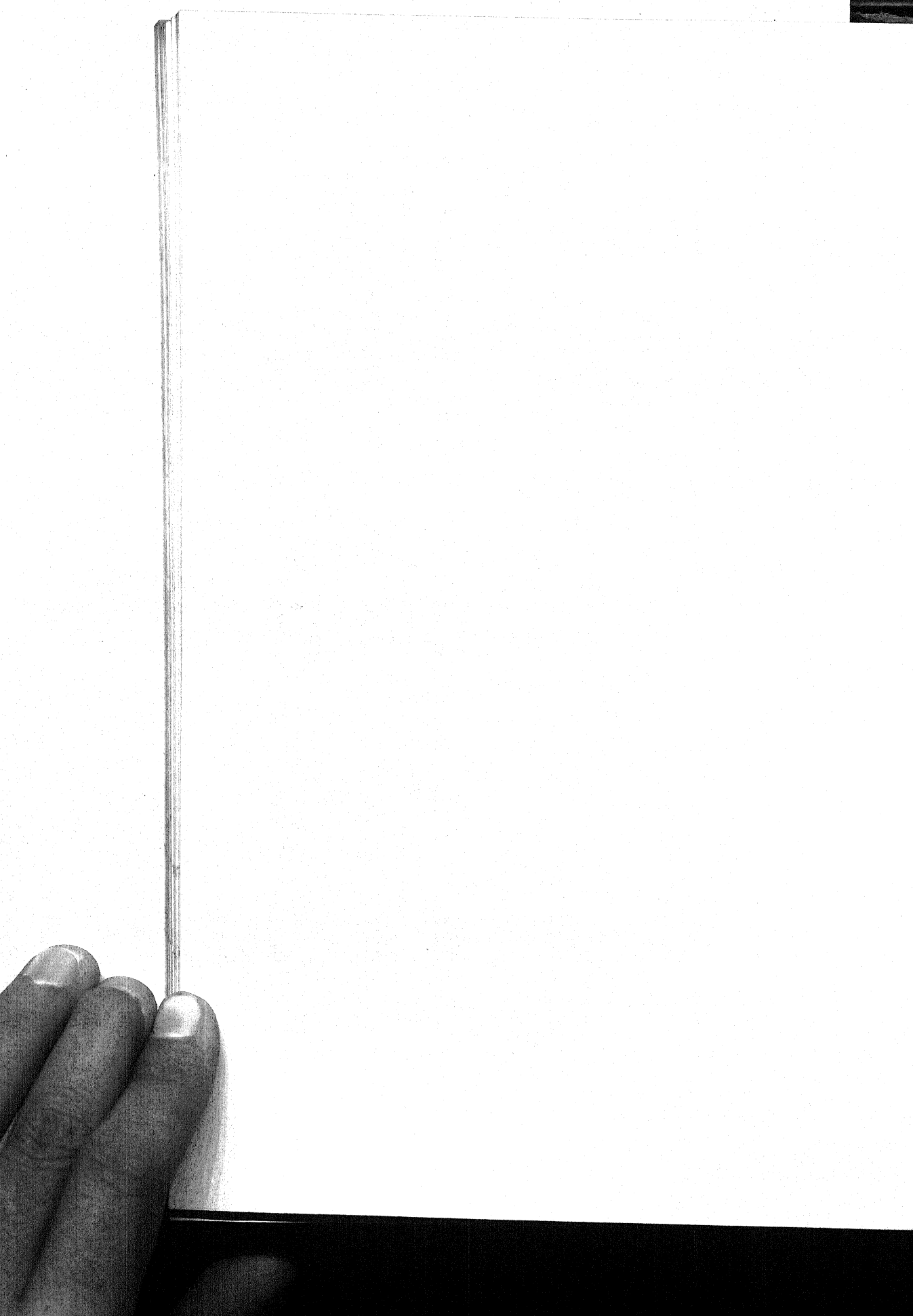

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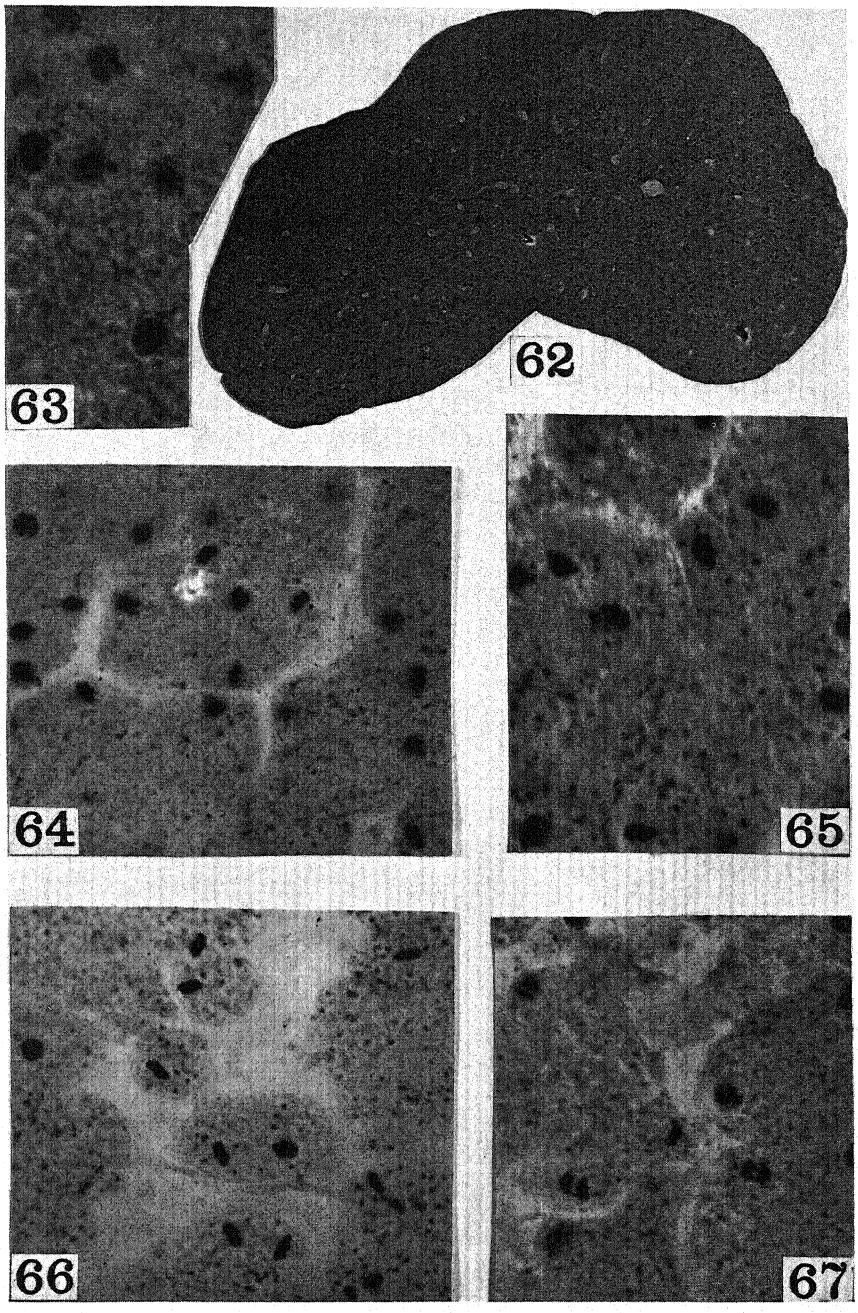

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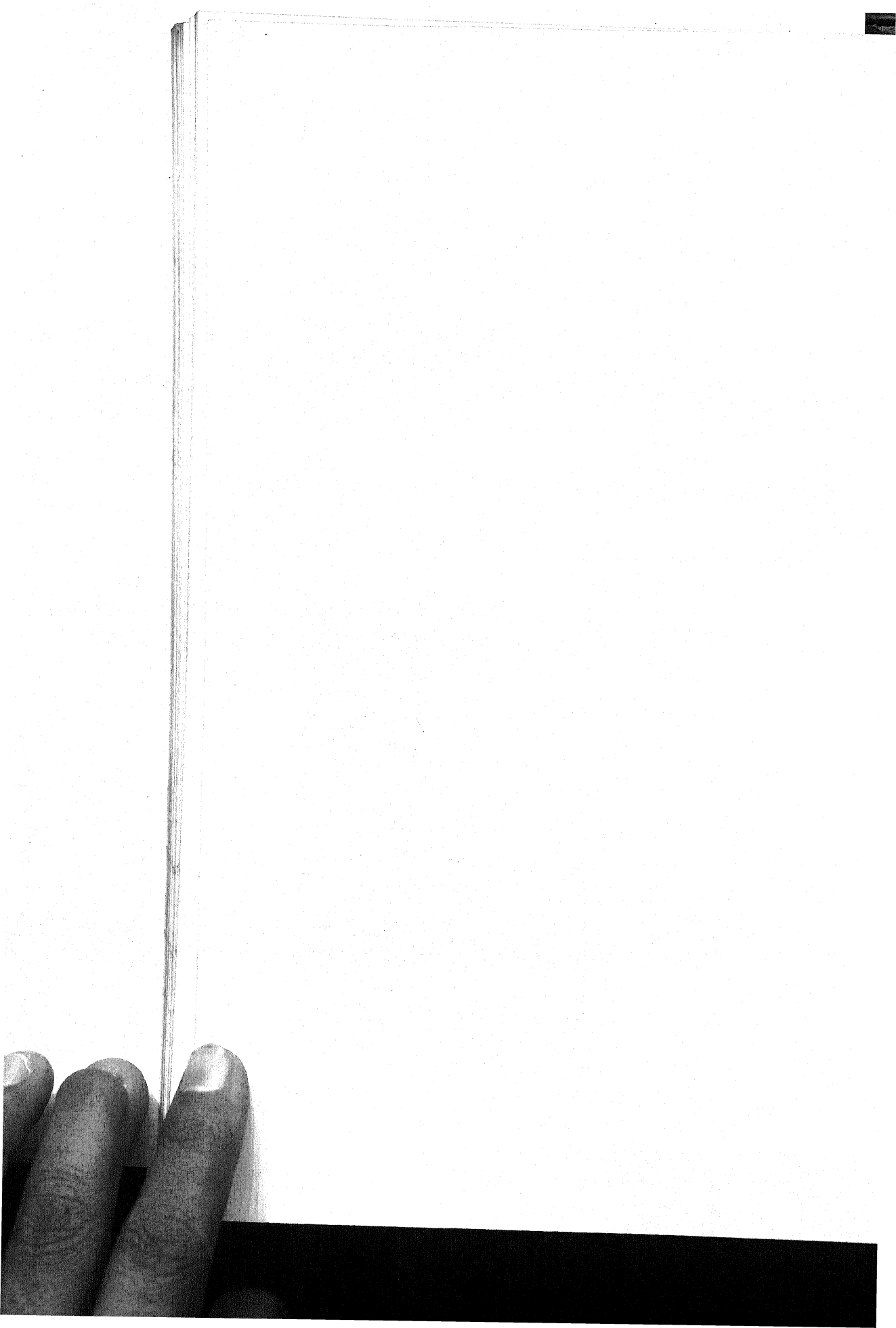

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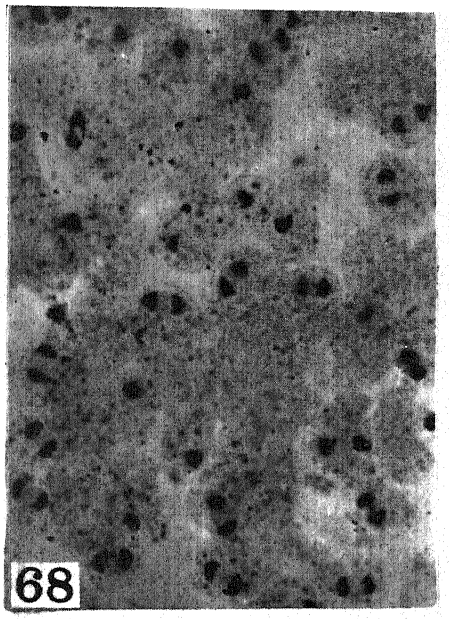

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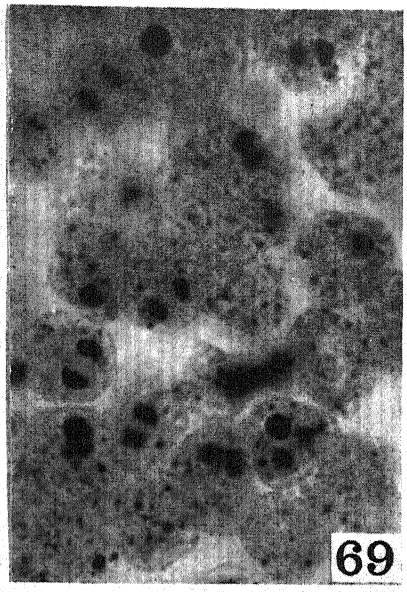




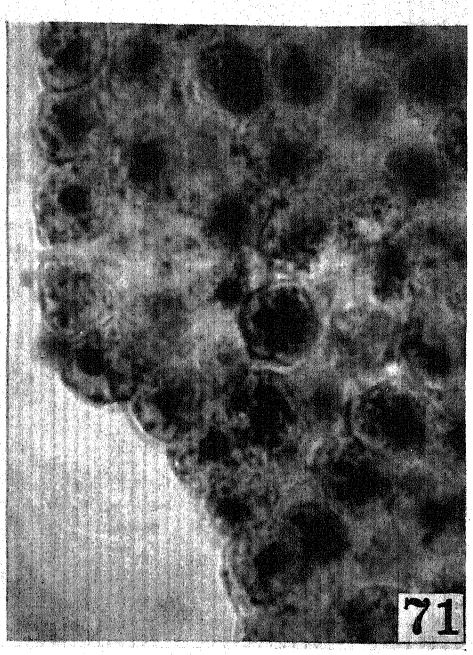
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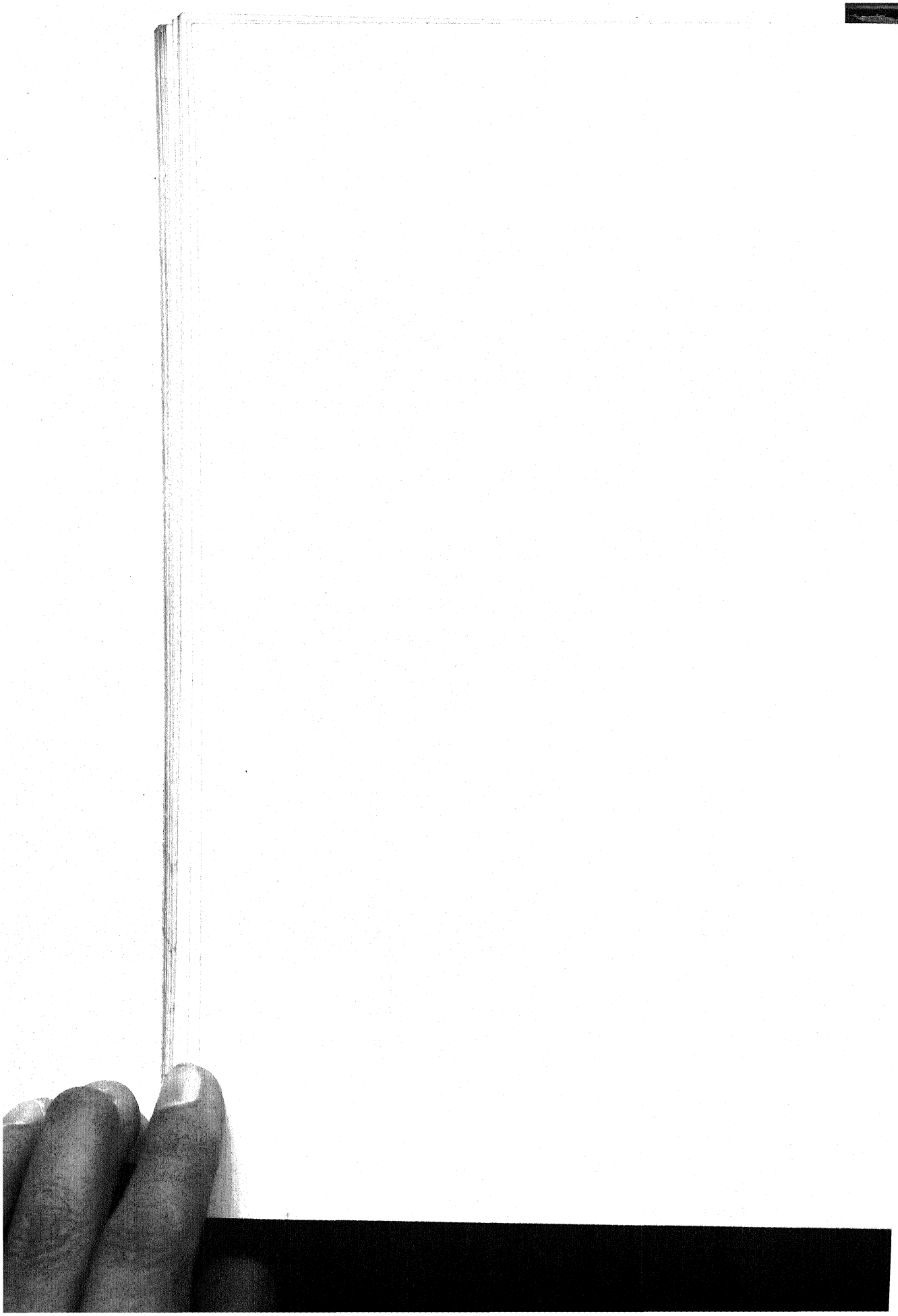


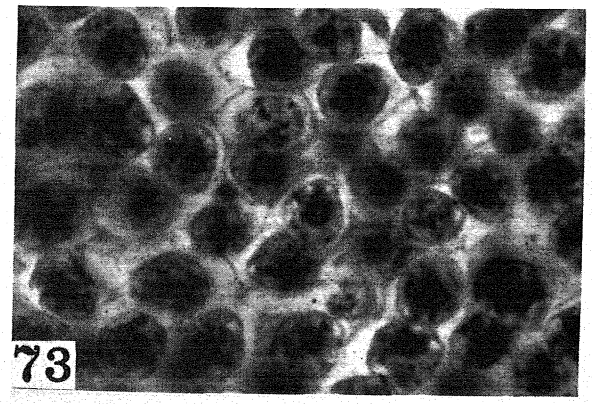


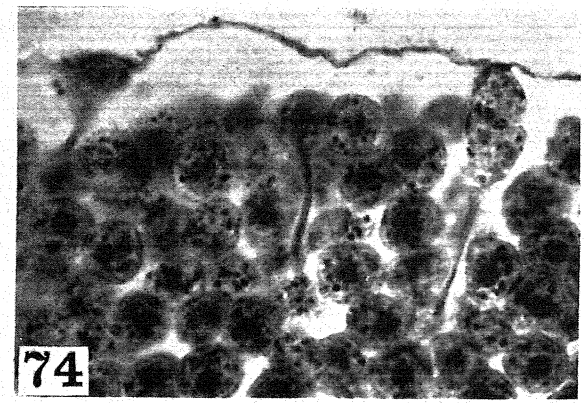


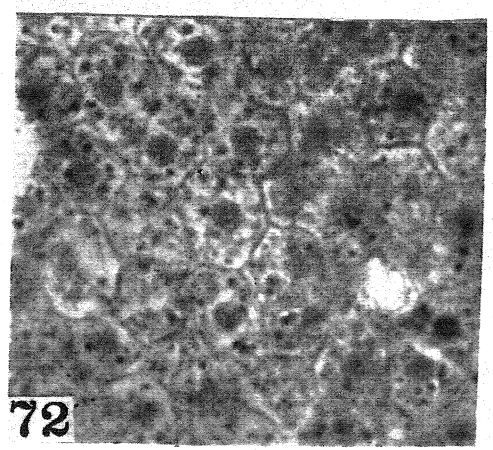


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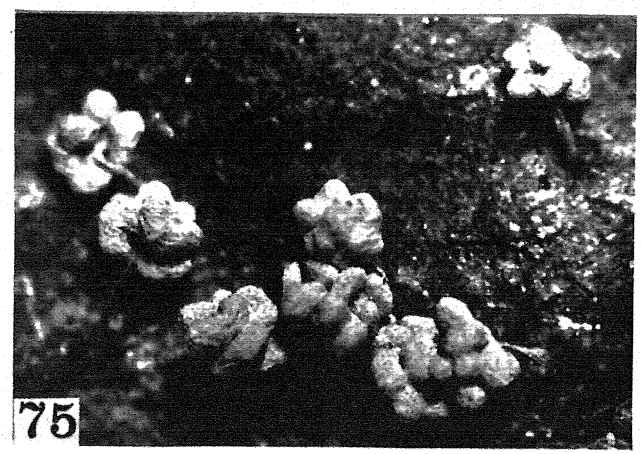

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PLATE XVI

FIG. 57. Tangential section of plasmodial layer after segmentation. Note the external membrane. × 65.

FIG. 58. Pillar stage. Note differentiation into stipe and sporangial head. × 5.

FIG. 59. Longitudinal section of a pillar showing apical cortical layer. × 70.

FIG. 60. Transverse section of a pillar showing reticulate protoplasmic structure and differentiated cortical layer. × 140.

FIG. 61. Median longitudinal section of stipe showing central granular core, ascending reticulate protoplasm, and stipe membrane. × 150.

PLATE XVII

FIG. 62. Tangential section of sporangial lobe showing homogeneous protoplasmic structure, evenly distributed nuclei, and lacunae in which the capillitium is being formed. × 115.

FIG. 63. Enlarged portion of figure 62 showing types of nuclei. × 3500.

FIGS. 64 and 65. Initiation of cytokinesis. Note furrows cutting through undifferentiated protoplasm. × 3000.

FIG. 66. Nuclei at metaphase. Note spindle and nuclear membrane. × 3000.

FIG. 67. Nuclei at anaphase. Note separation of chromosomes in clumps. × 3000.

PLATE XVIII

FIG. 68. Nuclei at anaphase. Note the connecting fibers. × 2800.

FIG. 69. Nuclei at anaphase enlarged to show the distinct centrosome. × 3500.

FIG. 70. Telophase. Note the length of the connecting fibers. × 3000.

FIG. 71. Uninucleate segments after the final cleavage at the edge of a section. × 3500.

PLATE XIX

FIG. 72. Intersporal membrane formed between hexagonal uninucleate segments. × 3500.

FIG. 73. Same stage as 72 but with protoplasts shrunken to bring out the continuous intersporal membrane. × 3500.

FIG. 74. Young rounded spores bounded by the peridium to which a lime-filled capillitial node is seen to be attached. × 3000.

FIG. 75. Habit of mature sporangia. × 8.

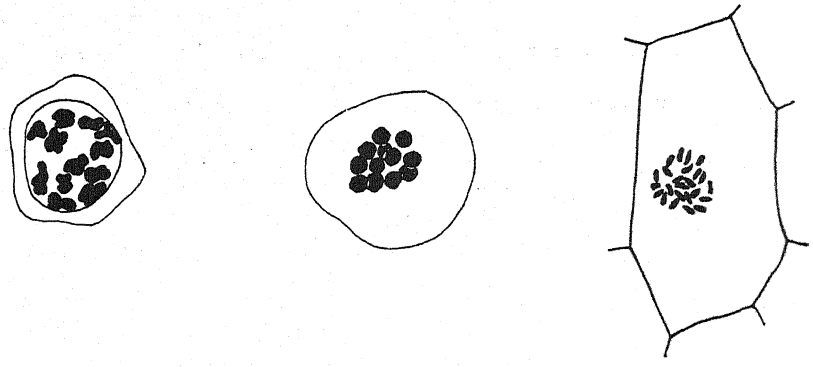
THE CHROMOSOMES OF *PIPER SUBPELTATUM*

DONALD A. JOHANSEN

(Received for publication August 12, 1930)

Some years ago the writer had occasion to embed some material of *Piper subpeltatum* L. (*Heckeria subpeltata* Kunth.) collected by Dr. Douglas H. Campbell at Buitenzorg, Java, in April, 1906. The material, killed in a chromic acid solution, was so extremely well preserved despite the long time it remained in 75% alcohol that it was possible to follow out many of the details of meiosis in the microsporocytes.

A vast amount of work has been performed in the study of the development and organization of the peculiar megagametophytes characterizing the different species of the Piperaceae, but in contrast it is worthy of note that the literature is apparently devoid of any paper of consequence upon microsporogenesis. It was with the idea of preparing such a paper that the material was worked up, but unfortunately it soon became evident that the material was insufficient to permit a thorough study.



1 2 3

TEXT FIGS. 1–3. 1, Diakinesis. 2, Equatorial plate of first meiotic mitosis. 3, Somatic equatorial plate. All × 2500.

In Dr. Campbell's material, the chromosomes were found to be of an extremely small size. Following diakinesis, the meiotic chromosomes are generally too much clumped together for one to be always certain of the number, but somatic figures are usually sufficiently clear. In diakinesis, the tetrad chromosomes stand out sharply and are easily countable (text fig. 1). At metaphase, as well as on the equatorial plate (text fig. 2), the number of

haploid chromosomes is twelve. Counts of several somatic figures give 24 as the diploid number (text fig. 3).

Upon checking up the literature a curious situation presented itself. Two earlier reports are available, but one of them is of extremely doubtful authenticity. Tischler (5) cites Palm (4) as giving the haploid number as 12, but a careful reading of that portion of Palm's paper devoted to *Piper subpeltatum* shows that he nowhere definitely cites either haploid or diploid numbers for this species. The error probably lies in a slightly careless reading of the second paragraph on page 48 of the paper in question: Palm is discussing another investigator's results on *Myricaria germanica*, and the phrase "die reduzierte Chromosomenzahl ist 12" refers to that species, and not to *Piper subpeltatum*. Furthermore, if Palm's fig. 11*a*, which represents one longitudinal section of a tetranucleate megagametophyte, be consulted, 8 haploid chromosomes may readily be counted on the micropylar spindle, and 16 on the chalazal, the latter number resulting from the earlier fusion of two haploid nuclei. Miss Gaiser (1) apparently perpetuates Tischler's error.

Previous to Palm's study, Johnson (3) had studied *P. subpeltatum*, but gives no chromosome numbers. Later, Häuser (2) reports that "Die Chromosomenzahl der generativen Kern . . . liess sich nicht exakt bestimmen, schätzungsweise beträgt sie etwa 20."

Consequently, the haploid chromosome numbers reported for *Piper subpeltatum* are now 8 (Palm, if an illustration is considered as constituting "publication"), 12 (Johansen), and ca. 20 (Häuser). The only fact which might provide an explanation for the discrepancy between these reports is that the material studied by these investigators came from widely separated localities. A thorough cytological study of the species is therefore highly desirable, and the material should be obtained from as many localities as possible.

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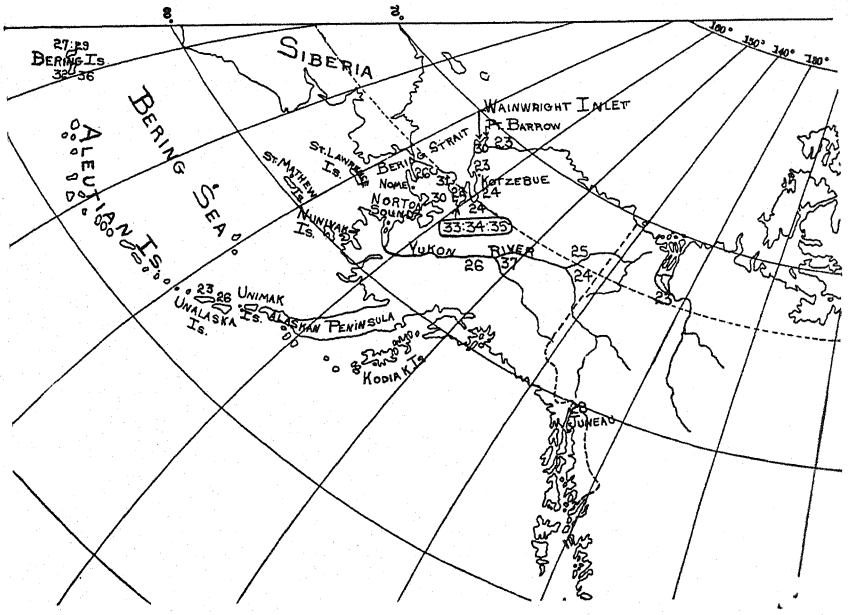
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GEOGRAPHIC AND TAXONOMIC DISTRIBUTION OF AMERICAN PLANT ARROW POISONS

RALPH H. CHENEY

(Received for publication August 23, 1930)

In a previous paper, the author (1923) described the ancient and modern use of vegetable species and derived substances as the source of arrow-tip poisons. The present article gives the facts and significance of the species utilized in various areas of the western hemisphere.

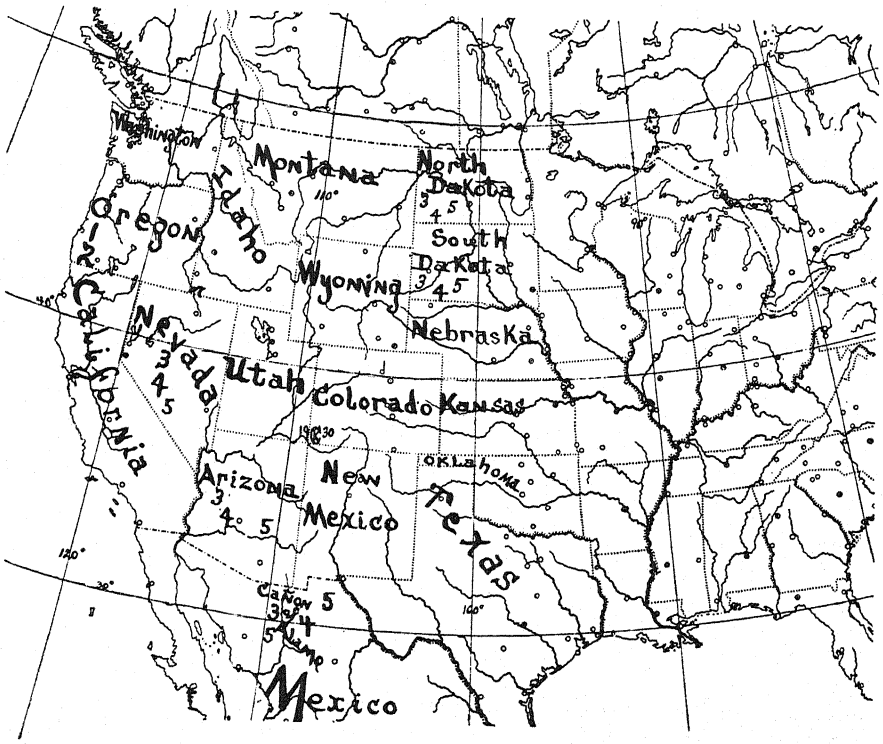


Numbers in the text figures indicate the species as numbered below.

TEXT FIG. 1. ALASKA. 22, *Anemone alpina* Linn.; 23, *A. narcissiflora* Linn.; 24, *A. parviflora* Michx.; 25, *A. patens* Linn.; 26, *A. Richardsoni* Hook.; 27, *Aconitum delphinifolium* DC.; 28, *A. Chamissonianum* Reichb.; 29, *Delphinium elatum* Linn.; 30, *Ranunculus hyperboreus* Rottb.; 31, *R. lapponicus* Linn.; 32, *R. nivalis* Linn.; 33, *R. pallasii* Schlecht.; 34, *R. purshii* Richards.; 35, *R. pygmaeus* Wahl; 36, *R. verticillatus* T. Kirk; 37, *Papaver nudicaule* Linn.

The Alaskan species (text fig. 1), so used, are limited primarily to the Ranunculaceous genus *Anemone*. The species utilized depends upon the abundance of the plant in the given geographical location (table 1). The actual use of such poisons has rapidly diminished in this area during recent

years. Formerly, certain species of *Aconitum*, *Delphinium*, *Ranunculus*, and *Papaver* were given a place as secondary ingredients occasionally included in the Alaskan poisonous arrow pastes. These species are also listed in the table with the areas indicated in which they were employed. The Alaskan flora is drastically limited ecologically and, in general, it is non-poisonous. The Eskimos have been successful in discovering the most toxic genus within their range and have commonly employed the roots of *Anemone* throughout the groups inhabiting the northern and northwestern territory. The tribes occupying the Canadian area have apparently never made use of plant poisons for such purposes although the flora includes more toxic species than Alaska. Since five species of *Anemone* were used in northern Asia, the secret of their application may have been passed northeastward to Alaska rather than northwestward from Canada.



TEXT FIG. 2. WESTERN UNITED STATES AND NORTHERN MEXICO. 1, *Cynanchum macrophyllum* Pers.; 2, *Conium maculatum* Linn.; 3, *Yucca glauca* Nutt.; 4, *Piscidia piscipula* Sarg.; 5, *Sebastiana Palmeri* Riley.

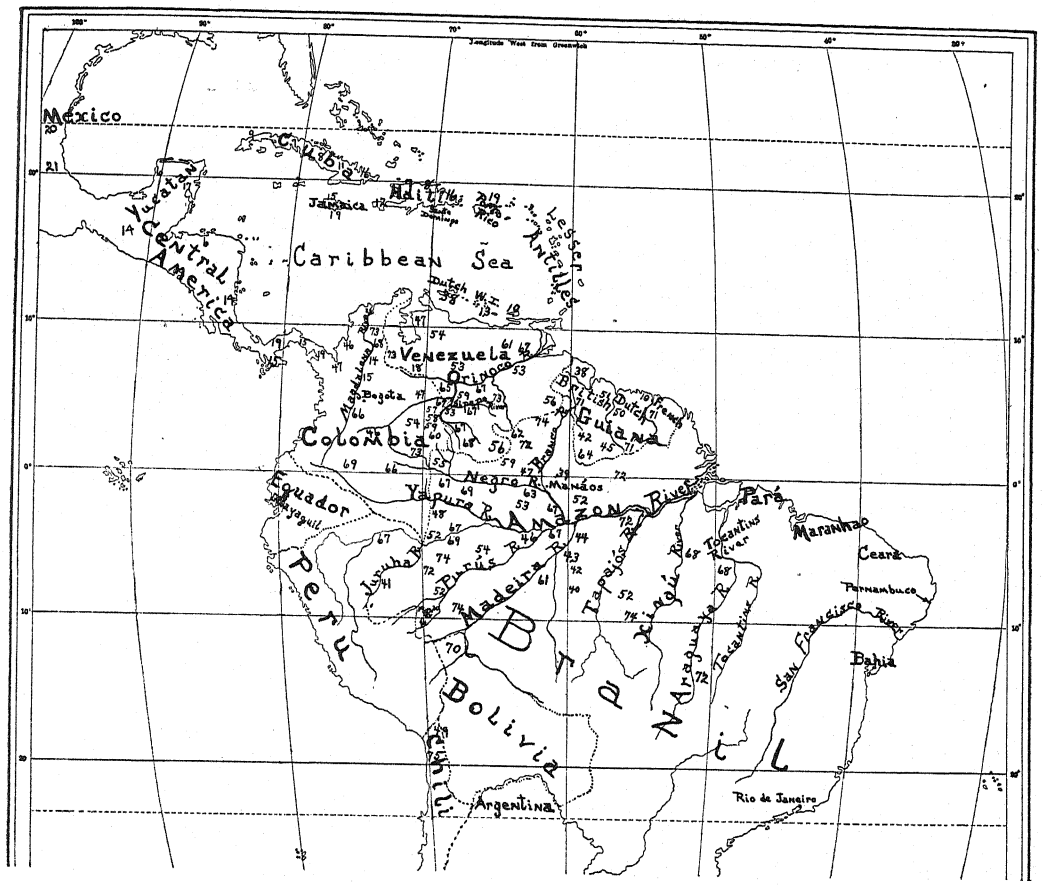
Although arrows were the common weapons throughout the area now comprising the United States (text fig. 2) and blow-guns were used in North Carolina, neither arrows nor darts were customarily poisoned. Le Mercier

(1638) reported that the Erie Indians, the Cat Nation, so-called because of the great abundance of wildcats in their territory, applied poisons to their arrows about 1653 during their warfare with the Iroquois but no record remains to indicate whether animal or plant sources were employed. The Indians of southwestern Oregon used the stems of *Cynanchum macrophyllum* Pers. and *Conium maculatum* Linn. in combination with animal or corpse substances in their preparations for smearing arrow tips. Mexican Indians, those of the Dakotas, Nevada, and Arizona, seem to have depended chiefly upon animal venom and putrefactive products in which they soaked their arrow heads. From the toxic flora at their command, they added only *Yucca glauca* Nutt. (formerly *Y. angustifolia* Pursh.), *Piscidia piscipula* Sarg., and a poisonous paste prepared from the latex of *Sebastiana Palmeri* Riley. Occasionally one or two other Euphorbiaceous species were utilized.

Central American tribes still employ plant poisons very commonly with criminal intentions but curiously have never developed the use of known vegetable poisons for arrow or dart application. The far-famed "camotillo" of the Mayas is reputed to be from a vegetable source. West Indian arrow poison preparations have been superseded by fire-arms but these plant poisons are now to be found among the drugs listed as commonly employed in the criminology of the island. The species include *Serjania nodosa* Radlk., *Serjania curassavica* Radlk., *Dieffenbachia Seguine* Scott (Dumb Cane), *Dioscorea trifida* Linn. (Cush-cush) and *Dioscorea hirsuta* Bl. (Tuber-yam).

South America has always been the center of many plant arrow poisons (text fig. 3) although their use is unknown throughout the southern half of the continent. In central, northwestern, and northern South America, both arrows and blow-guns with darts are still poisoned and used in hunting and warfare. The Amazon, Magdalena (primarily animal poisons), and Orinoco river basins and the areas of British, Dutch, and French Guiana are the territories where the vegetable arrow poisons are most abundant. The one of greatest potency employed over wide areas is the well-known Curare obtained primarily from various species of the bindweed *Strychnos*, especially from *Strychnos toxifera* Schomb. ex Benth., *S. Gubleri* Planch., and *S. Castelnaei* Wedd.

The preparation of this poison differs in its minor ingredients and several types are described by Lewin (1923). Three principal forms of Curare are known,—Carriços (clay pots), Tubocurare (bamboo internodes) or Paracurare (*i.e.* exported from Para), and Gourd Curare which possesses the greatest potency. The Gourd Curare of the Piraoa Indians in the Sipapo river district makes the most effective curare in South America and it is sought by other tribes a thousand miles away who send messengers with gifts to obtain that particular form. Only Tubocurare (Paracurare) is exported at present. Curare was first carried to Europe from British Guiana in 1595 by Sir Walter Raleigh, and curiously enough, at the present time some of the Ecuadorian Indians smear their arrow tips with curare which has been



TEXT FIG. 3. CENTRAL AMERICA, WEST INDIES, SOUTH AMERICA. 6, "Camotillo"; 7, *Serjania nodosa* Radlk.; 8, *Serjania curassavica* Radlk.; 9, *Dieffenbachia Seguine* Scott; 10, *Dioscorea trifida* Linn. f.; 11, *D. hirsuta* Bl.; 12, *D. alata* Linn.; 13, *D. cayenensis* Lam.; 14, *Hippomane mancinella* Linn.; 15, *Hura crepitans* Linn.; 16, *Pedilanthus tithymaloides* Poit; 17, *Jacquinia armillaris* Jacq.; 18, *J. carcasana* H. B. & K.; 19, *J. arborea* Vahl; 20, *Nicotiana obtusifolia* Mart.; 21, *N. rustica* Linn.; 38, *Dioscorea cayenensis* Lam.; 39, *Piper geniculatum* Sw.; 40, *Ficus atrox* Mart. ex Miq.; 41, *Anomospermum grandifolium* Eichl.; 42, *A. lucidum* Miers.; 43, *A. ovatum* Miers.; 44, *A. reticulatum* Eichl.; 45, *A. Schomburgkii* Miers.; 46, *Cocculus amazonum* Mart. ex Herberger; 47, *C. macrophyllus* St. Hil. & Tul.; 48, *Guatteria venificiorum* Mart.; 49, *Colliguaja odorifera* Molina.; 50, *Euphorbia cotinoides* Miq.; 51, *Spigelia fruticulosa* Lam.; 52, *Strychnos brasiliensis* Mart.; 53, *S. Castelnaei* Wedd.; 54, *S. cogens* Benth.; 55, *S. Crevauxii* Planchon; 56, *S. Curare* H. Baill.; 57, *S. depauperata* H. Bn.; 58, *S. Gardneri* A. DC.; 59, *S. Gubleri* Planch.; 60, *S. guianensis* Mart.; 61, *S. hirsuta* Spruce; 62, *S. Jobertiana* H. Bn.; 63, *S. nigricans* Prog.; 64, *S. pedunculata* Benth.; 65, *S. rubiginosa* A. DC.; 66, *S. subcordata* Spruce; 67, *S. toxifera* Schomb. ex Benth.; 68, *S. triplinervia* Mart.; 69, *S. yapurensis* Planch.; 70, *Tabernaemontana acuminata* Muell.-Arg.; 71, *T. speciosa* Poir.; 72, *T. solanifolia* A. DC.; 73, *Capsicum annum* Linn.; 74, *Nicotiana acutifolia* A. St. Hil.; 75, *N. Miersii* Remy.

imported from Germany. The curare on the United States market is also imported from Germany. Curare is seldom, if ever, prepared entirely from the stems of one or several of the eighteen South American species of *Strychnos* although the efficiency of the poison is due almost completely to the action of the alkaloids—curine and curarine—which paralyze the motor end-plates without affecting the excitability of the sarcoplasm, and may cause death without convulsions or pain. It is of distinct physiological interest to notice the great difference between this action of the South American species of *Strychnos* and the numerous African and Asiatic ones which contain strychnine and brucine and act as tetanizers on the central nervous system. The minor components invariably added differ widely in accordance with the tribal superstitions and the local flora. Plants frequently employed include numerous species of the Amarantaceae, Araceae, Euphorbiaceae, Loganiaceae, Menispermaceae, Phytolaccaceae, Piperaceae, Solanaceae, and Urticaceae. The Menispermaceae are especially prized as secondary ingredients in the preparation of violent poisons and are usually omitted from material to be used in hunting birds and other minor game. The Piperaceae serve to stimulate the wound and prevent blood coagulation. Dr. W. L. Schurz reports a South American tree called "assacú" possessing an astringent and poisonous sap, but I have been unable to ascertain its botanical identity.

The taxonomic relationships of American plant arrow poison species, the part used, and the irritant action on animal organisms, are shown in table 1.

TABLE 1

Classification	Geographic Distribution of Species, and Part Used	Alkaloid Present: Irritant Property or Action
Thallophyta, Bryophyta, Pteridophyta and Gymnospermae are not used.		
Araceae		
Dieffenbachia Seguine Scott (Dumb Cane)	Stem and root extract. West Indies (Cuba).	Narcotic: Gastric and kidney irritant.
Liliaceae		
Yucca glauca Nutt.	Stem and leaf extract. Dakotas, Nevada, Arizona, Mexico.	Diuretic.
Dioscoreaceae		
Dioscorea alata Linn. (Winged-stalked or White Yam)	Stem and tuber. West Indies.	Dioscorine. Paralyzes the central nervous system. Acts like picro-toxin.
D. cayenensis Lam.	Stem and tuber. Dutch West Indies and British Guiana.	
D. hirsuta Bl. (Tuber-yam)	Stem and tuber. West Indies.	

TABLE I (*Continued*)

Classification	Geographic Distribution of Species, and Part Used	Alkaloid Present: Irritant Property or Action
D. trifida Linn. (Cush-cush)	Stem and tuber. Dutch W. I. and Guiana.	
Piperaceae		
Piper geniculatum Sw.	Fruit and root. South America.	Tissue irritant.
Moraceae		
Ficus atrox Mart. ex Miq.	Stem-bark: Fruit. Brazil.	
Urticaceae		
Numerous So. American species	Leaves. So. America.	Tissue inflammation.
Amarantaceae		
Numerous So. American species	Inflorescence: Seeds. South America.	Thickener.
Phytolaccaceae		
Numerous So. American species	Fleshy roots: Fruits. South America.	Phytolacca (slightly narcotic).
Ranunculaceae		
Anemone	Roots: Leaves. Alaska.	Anemonin ($C_{15}H_{12}O_6$). Anemonic Acid ($C_{15}H_{14}O_7$)
A. alpina Linn.	Kotzebue Sound.	
A. narcissiflora Linn.	Kotzebue Sound. Pt. Barrow to Mackenzie River, Unalaska Island.	
A. parviflora Michx.	Kotzebue Sound.	
A. patens Linn.	Fort Yukon. Whole fresh plant in flower.	Lacrymation in mammals. Diuresis. Gastric and intestinal irritant (pains). Fever. Debility.
A. Richardsoni Hook.	Unalaska, Kotzebue Sound, Yukon River.	
Aconitum delphinifolium DC.	Roots. Bering Island.	Unknown.
A. Chamissonianum Reichb.	Roots. Juneau, Alaska.	Possibly aconitine.
Delphinium elatum Linn.	Roots and Leaves. Bering Island.	
Ranunculus hyperboreus Rottb.	Entire green plant in fruit or the roots and leaves. Norton Sound to Wainwright Inlet.	Anemonol (oil), stupor and paralyzing narcotic with convulsions. Anemonin, similar to action of anemonol but without convulsions.
R. lapponicus Linn.	Roots and Leaves. Kotzebue Sound.	
R. nivalis Linn.	Roots and Leaves. Bering Island.	
R. pallasii Schlecht.	Roots and Leaves. Kotzebue Sound.	
R. purshii Richards	Roots and Leaves. Kotzebue Sound.	

TABLE I (*Continued*)

Classification	Geographic Distribution of Species, and Part Used	Alkaloid Present: Irritant Property or Action
R. pygmaeus Wahl.	Roots and Leaves. Kotzebue Sound.	
R. verticillatus T. Kirk.	Roots and Leaves. Bering Island.	
Leguminosae		
Piscidia piscipula Sarg. (Syn. *P. Erythrina* Linn.)	Fruits. Nevada, Arizona, Mexico.	Dizziness: Coma.
Euphorbiaceae		
Colliguaja odorifera Molina.	Latex. Northern Chili.	
Euphorbia cotinoides Miq.	Latex, roots. Guiana.	Euphorbium (purgative). Euphorbin (arterial sedative).
Hippomane mancinella Linn. "Manchineel"	Fruit. Latex (used by Caribs). West Indies, Central America and Columbia.	Poisonous.
Hura crepitans Linn. "The sand-box tree"	Latex. Panama Canal Zone, Jamaica, San Salvador, So. America.	Poisonous milky latex. Seed oil: purgative.
Pedilanthus tithymaloides Poit.	Latex. Antilles (W. I.)	
Sebastiana Palmeri Riley.	Latex. Cañon of Alamo, Sonora, Mexico.	
Sapindaceae		
Serjania curassavica Radlk.	Root and oil in which fruits have been cooked. West Indies.	Timboïne: Narcotic.
S. nodosa Radlk.	Root and oil as above. W. I.	Timboïne: Narcotic.
Umbelliferae		
Conium maculatum Linn. "Poison hemlock"	Entire fresh plant except root. South W. Oregon. Effect: Spinal Irritant. Paralysis of appendages. Paralysis of respiratory muscles resulting in gradual asphyxiation.	Contains alkaloids (conia $C_8H_{15}N$; methylconine $C_8H_{14}NCH_3$; conydrine $C_8H_{17}ON$; paraconine $C_8H_{15}N$ isomeric with conia. Oil of conium $C_8H_{16}N_2O$. Conic Acid).
Theophrastaceae		
Jacquinia armillaris Jacq.	Crushed fruit. Yucatan.	Poisonous.
J. arborea Vahl	Panama Canal Zone: W. I.: Jamaica: Porto Rico: No. South America.	
J. carcasana H. B. & K.	Venezuela.	
Papaveraceae		
Papaver nudicaule Linn.	Entire plant. Alaska.	Possibly sedative.
Menispermaceae		
(Characteristically, this family has an antiseptic action and thereby inhibits mold and bacterial growth, hence possibly reducing the rate of deterioration of curare).		

TABLE I (*Continued*)

Classification	Geographic Distribution of Species, and Part Used	Alkaloid Present: Irritant Property or Action
Anomospermum grandifolium Eichl.	Roots. Brazil.	
A. lucidum Miers.	Roots. Guiana.	
A. ovatum Miers.	Roots. Brazil.	
A. reticulatum Eichl.	Roots. Brazil.	
A. Schomburgkii Miers.	Roots. Guiana.	
Cocculus amazonum Mart. ex Herberger	Stem bark. Brazil, N. W. South America.	
C. macrophyllus St. Hil. and Tul.	Stem bark. Brazil, N. W. South America.	
Anonaceae		
Guatteria venificiorum Mart.	Stalked berries (fruits). Brazil.	
Loganiaceae		
Spigelia fruticulosa Lam.	Guiana.	
Strychnos (18 species)	South America. Stem and bark.	Curine and Curarine paralysis.
S. brasiliensis Mart.	Latex of stem and bark. Brazil.	Paralysis.
S. Castelnaei Wedd.	Stem and bark latex. Valley of Orinoco and Amazon Rivers.	Curarine ($C_{19}H_{26}ON_2$) Paralysis of nerve endings.
S. cogens Benth.	Stem and bark latex. Brazil, No. and N. W. So. Amer.	
S. Crevauxii Planch.	As above.	
S. Curare H. Baill.	"	
S. depauperata H. Bn.	"	
S. Gardneri A. DC.	"	
S. Gubleri Planch.	"	
S. guianensis Mart.	"	Used especially for weak curare.
S. hirsuta Spruce	"	
S. Jobertiana H. Bn.	"	
S. nigricans Prog.	"	
S. pedunculata Benth.	"	
S. rubiginosa A. DC.	"	
S. subcordata Spruce	"	
S. toxifera Schomb. ex Benth.	Stem and bark. Valley of Orinoco and Amazon Rivers.	Used for strong curare.
S. triplinervia Mart.	Latex of stem and bark. Brazil, No. and N. W. So. Amer.	
S. yapurensis Planch.	As above.	
Apocynaceae		
Tabernaemontana acuminata Muell. Arg.	Bark. Bolivia.	Cardiac poison.
T. speciosa Poir.	Bark. Guiana.	
T. solanifolia A. DC.	Bark. Brazil.	

TABLE 1 (*Continued*)

Classification	Geographic Distribution of Species, and Part Used	Alkaloid Present: Irritant Property or Action
Asclepiadaceae		
Cynanchum macrophyllum Pers.	Fleshy stems. Seeds. Southwestern Oregon.	Poisonous. Strangling effect.
Solanaceae		
Capsicum annum Linn.	Fruits. N. W. So. Amer.	Tissue irritant.
Nicotiana acutifolia A. St. Hil.	Dried leaves. Seeds. Brazil.	Nicotine.
N. Miersii Remy	Chili.	
N. obtusifolia Mart.	Mexico.	
N. rustica Linn.	Mexico.	

The geographic distribution of the use of vegetable arrow poisons in the western hemisphere is shown by the accompanying figures (text figs. 1, 2, 3).

SUMMARY

1. The utilization of vegetable poisons for anointing weapons (arrows, darts, bullets) is not entirely coincident with the presence of a toxic flora.

2. The most poisonous species indigenous to a given area have been applied successfully by the natives of distantly separated geographic localities.

3. The employment of widely separated taxonomic species suggests an independent development of American arrow poison preparations.

4. The predominating toxic flora with respect to the number of species and abundance of individuals of that species in the western hemisphere is to be found in the northern half of South America which is also the center of the use of plant arrow poisons.

5. Examination of the western hemisphere flora utilized in the preparation of poisons for weapons reveals the facts that at least 21 families of the Angiospermae are involved and that the most numerous and potent species belong to the curarizing genus *Strychnos* (Loganiaceae).

6. In most instances, the knowledge that active principles are usually more concentrated in some definite portion of the plant was apparently noted in the past and seems to be appreciated at present, inasmuch as the natives generally utilize a specific part, and only rarely employ the entire plant.

7. The fact that alkaloids and the potency of other irritants gathered during certain stages of the development of the plant vary greatly was, and still is, partly understood in practice if not in theory, where these poisons are still employed; because in certain instances, the raw materials are collected and prepared only in accordance with such a principle. This fact is, of course, well recognized in the modern cultivation of drug plants for medicinal purposes.

8. The effective principles vary as widely in their chemical composition as their taxonomic source. The chief ingredients of these plants, known to cause physiological disturbances when injected into animals, are dioscorine, phytolacca (narcotic), anemonin, anemonic acid, anemonol, euphorbium, euphorbin, timboïne, conia, methylconine, conydrine, paraconine, oil of conium, conic acid, curine, curarine, and nicotine.

9. The physiological action of the different plant arrow poisons also shows great diversity, and in many instances causes injury simultaneously to many parts of the body because of the compound nature of the preparations. The more common effects are narcosis, gastric and kidney irritation including diuresis, general tissue inflammation, lacrymation, general debility, intestinal pains, convulsions, purgation, arterial sedation, paralysis of peripheral nerve endings, and central nervous system paralysis.

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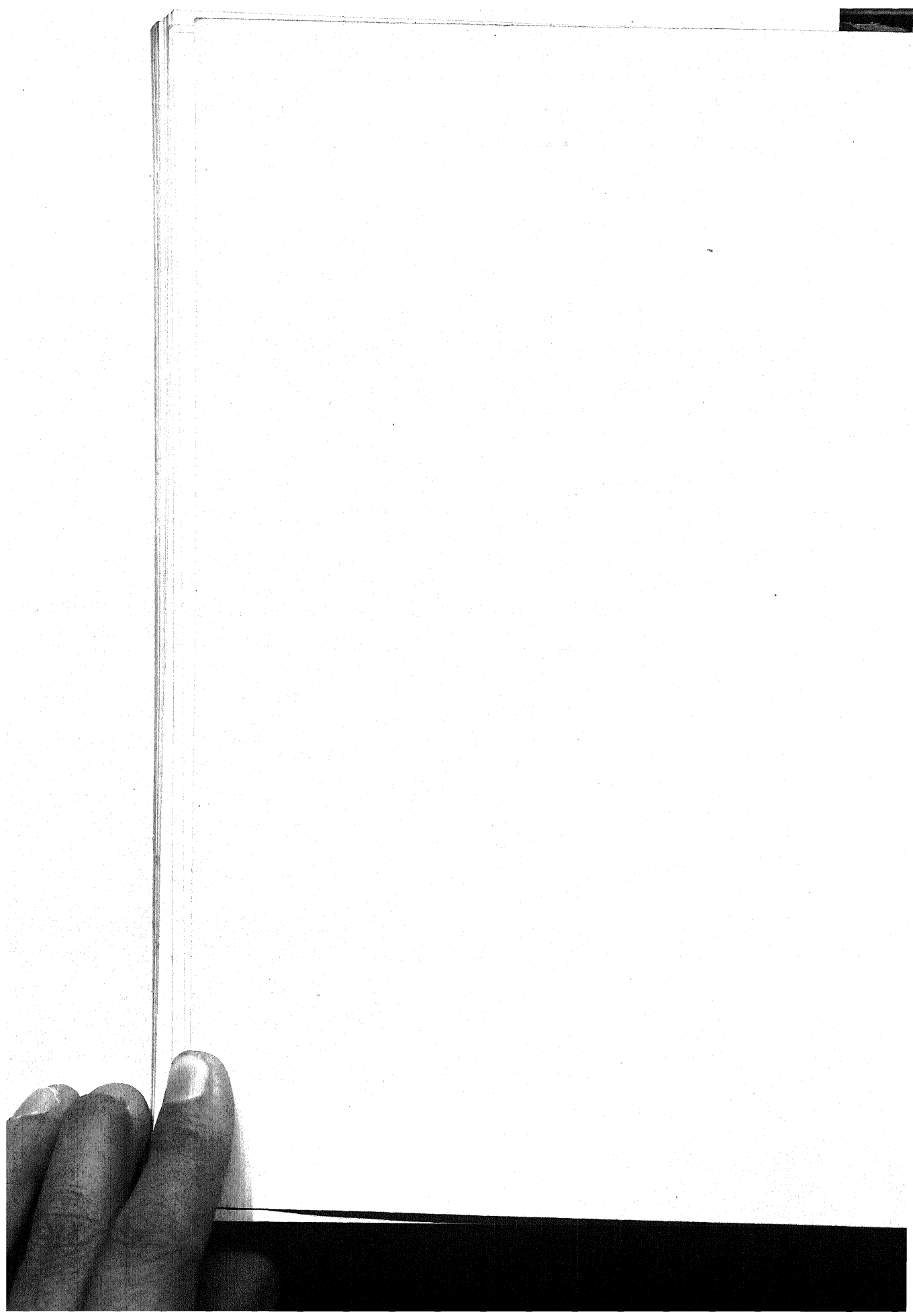
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THE VASCULAR ANATOMY OF THE FLOWER WITH REFUTATION OF THE THEORY OF CARPEL POLYMORPHISM [1]

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The flower morphologically is a determinate stem with appendages, and these appendages are homologous with leaves. This commonly accepted view of the nature of the flower is sustained by its anatomical structure. Flowers, in their vascular skeletons, differ in no essential way from leafy stems. They are, however, in this respect often more complex than most stems and their structure more obscure, because of the frequently large number of appendages and the crowding of these organs with resulting fusion, because of the reduction which many groups have undergone, and because of the determinate nature of the stem.

The pedicel and the receptacle have typical stem structure, with a normal vascular cylinder, and each of the appendages has traces in every way similar to those of leaves. These appendage traces are derived from the receptacular stele exactly as leaf traces are derived in typical stems. In many cases the vascular cylinder is simple, broken only by the gaps of these traces; more often it consists of discrete strands, as in the so-called herbaceous type of stem. In either case, when the floral organs are numerous and closely placed, the gaps of the traces break the receptacular stele into a meshwork which may be very complex.

This complexity and the obscurity of fundamental structure due to concrescence and reduction often render the interpretation of the vascular skeleton of the flower difficult. The great range of form and of modification in the flower adds still further to the difficulties. It is obvious that a real understanding of the vascular skeleton of the flower demands an acquaintance with the anatomy of flowers throughout Angiosperms. And this understanding of the anatomy of the flower—a specialized stem—must be based, certainly, upon a thorough acquaintance with the anatomy of leafy

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stems. The use of anatomical structure to determine fundamental morphology in the flower can only be sound when it is based upon a broad knowledge of the anatomy of stems and of the flower in its vast variety of form.

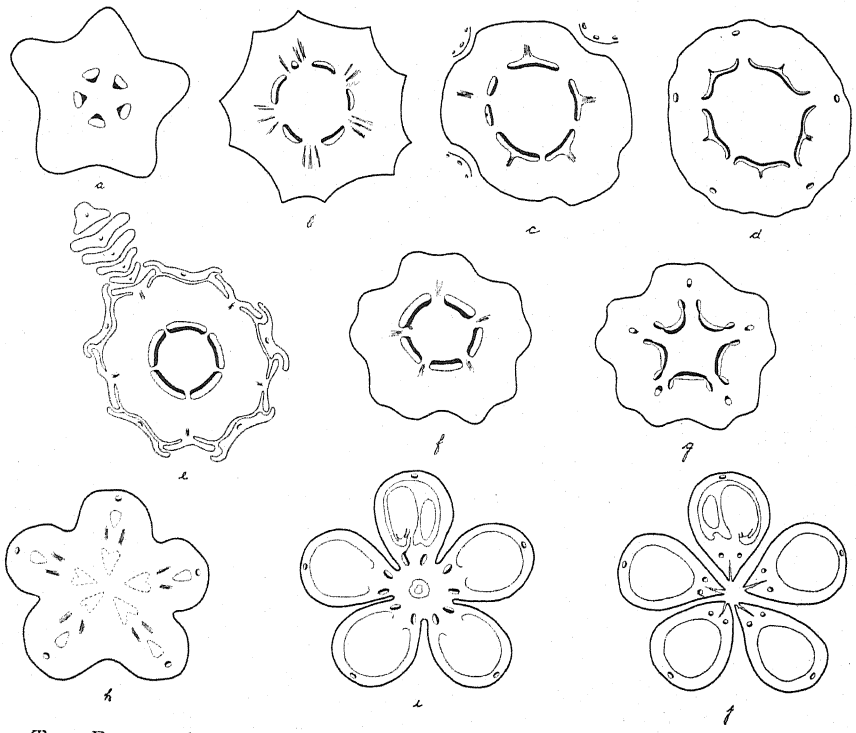
Taxonomy and comparative morphology have in large measure determined the structural nature of the flower. Anatomy has thus far aided only in the solution of certain puzzling conditions; for example, the fusion and reduction in the orchid flower. However, anatomy can provide much evidence additional to that already at hand from external form, evidence which is of the greatest value in the determination of the fundamental morphology of the flower.

This evidence, produced from the internal structure of the flower, from its slow-to-change skeleton, strongly reinforces in most cases the opinions commonly held concerning the morphology of the flower. Extensive studies in flower anatomy make it clear that the evidence adduced by general morphology, taxonomy, ontogeny (to some extent), and vascular anatomy goes as a whole hand in hand, and that the generally accepted views concerning the underlying nature of the flower are substantiated by evidence from all of the fields.

As a basis for this discussion, let us first examine some anatomically simple and typical flowers, one from the Dicotyledons and one from the Monocotyledons.

Aquilegia (Ranunculaceae, text fig. 1) serves well for the first group. In the pedicel are five bundles (fig. 1*a*). These unite at the base of the flower, forming an unbroken cylinder. The sepal traces are then given off (fig. 1*b*), three to each sepal, the three usually from one gap. (Occasional sepals have two or three gaps associated with each sepal.) The traces to the petals, one to each, are next given off (fig. 1*c*), just before the sepal gaps close; then the stamen traces pass off, one to each organ, in several whorls (fig. 1*d*, *e*). Above the uppermost whorl of stamens the vascular cylinder becomes complete again. Then the midrib traces of the carpels are given off (fig. 1*f*), followed by the pairs of lateral traces to each carpel. These lateral traces are derived from the sides of the gaps formed by the midrib traces (fig. 1*g*), and their gaps therefore merge with those of the midrib traces. The lateral traces immediately become twisted and pass out into the carpel inverted. Figure 1*h* shows the condition in the receptacle just above the outward passage of the lateral carpel traces. The base of the loculus of each carpel is seen with the midrib trace to the outside and the pair of laterals to the inside. The vascular cylinder is seen as five masses of less strongly developed tissue (shown by dotted lines), phloem chiefly, with some weak xylem. This tissue becomes a cylinder (fig. 1*i*, center), which grows smaller and weaker toward the rounded top of the receptacle and fades out. This behavior of degenerate vascular tissue is similar to that often seen in the vascular cylinder of determinate leafy stems.

Figure 1*i* shows the carpels nearly free from the receptacle, with their three-bundle supply. Figure 1*j* (above the receptacle) shows cross sections of the typical follicles with their midrib, or dorsal, bundles, and their pairs of inverted lateral, or ventral, bundles.

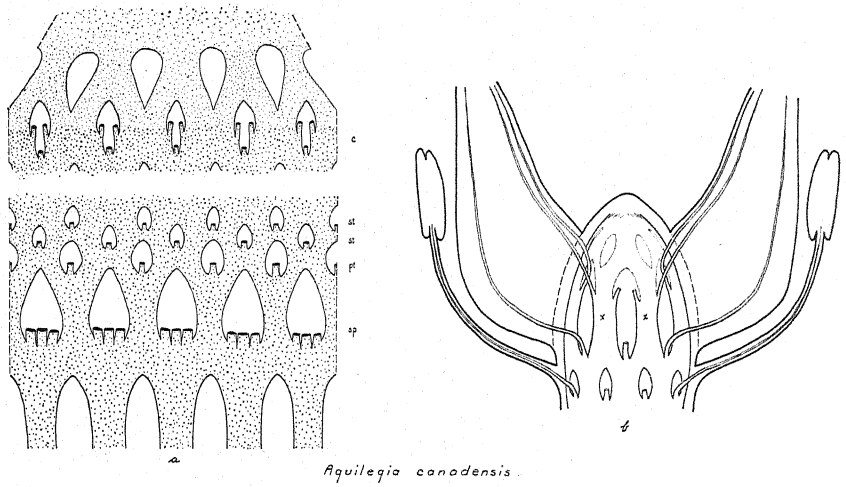


TEXT FIG. 1. *Aquilegia canadensis.* Cross sections of the flower at successive levels: *a*, the peduncle; *b*, the sepal traces departing in 3's, from a single gap usually; *c*, the petal traces departing; *d*, the petal traces far out in the receptacle, their gaps evident, and the traces to the first whorl of stamens departing; *e*, the traces to the last whorl of stamens departing, their gaps evident (9 whorls of 5 stamens omitted); *f*, the dorsal traces of the carpels departing; *g*, the ventral traces of the carpels departing in pairs from the sides of the dorsal trace gaps; *h*, the dorsal carpel traces far out, the ventral traces running outward, the bases of the loculi evident, the stele of the receptacle continuing as strands; *i*, the carpels nearly free, the pairs of ventral traces half-inverted, the receptacle bundles united into a cylinder; *j*, the carpels, showing dorsal and ventral bundles, the latter inverted.

Text figure 2 will make the structure of this flower more clear. In figure 2*a* the vascular cylinder only is shown, and is represented as split down one side and spread out. The position and relations of the traces and gaps are evident, also the weak terminal portion of the cylinder. (Several rows of stamens are omitted, only two being shown.)

Figure 2*b* represents a side view of the top of the floral cylinder to show the method of origin of the traces to the carpel (the twisting of the ventrals

is omitted) and the continuation of the degenerate cylinder tip beyond the uppermost traces. (The conical nature of the top of the receptacle is exaggerated to show more clearly this important point.) Under the polymorphism theory all this weaker tip of the cylinder is merely vascular tissue which is superfluous, or useless to the flower—is left over—and is, therefore, teleologically, "discarded into the pith." (In successive cross sections of the rounded top of the receptacle it of course appears to pass into the pith.)



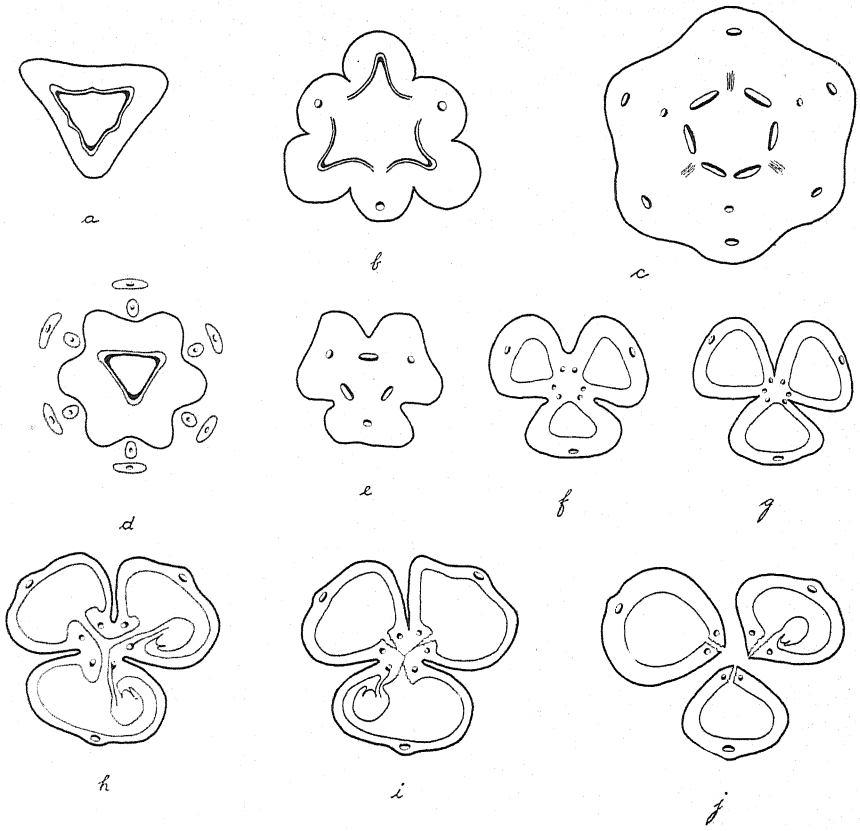
TEXT FIG. 2. *Aquilegia canadensis.* *a*, the vascular cylinder of the receptacle represented as split longitudinally and spread out, showing the position, number, and relations of the traces and gaps of the organs, and the continuation of the cylinder beyond the carpel traces. Nine rows of stamens have been omitted. *sp*, sepal; *pt*, petal; *st*, stamen; *c*, carpel. *b*, lateral view of top of vascular cylinder of receptacle, showing carpel traces and gaps and tip of cylinder.

In *Scheuchzeria* (Juncaginaceae), the pedicel has an unbroken cylinder (text fig. 3*a*). This is broken by the first three perianth traces (fig. 3*b*), then successively by the second whorl of three and by the two whorls of stamen traces (fig. 3*c*). The vascular cylinder then closes, to be broken soon again by the dorsal carpel traces (fig. 3*e*). The three broad bundles remaining each break into two (fig. 3*f*), and each pair forms two ventrals of adjacent carpels, becoming inverted at once (fig. 3*g*). The carpels (fig. 3*j*) are similar to those of *Aquilegia*.

In *Scheuchzeria* the vascular cylinder does not extend beyond the point of departure of the ventral carpel traces; the bundles remaining after the dorsal traces of the carpels pass out divide, a little higher up, and become ventral carpel traces. Thus the vascular tissue is all used up in the formation of the uppermost traces, just as in determinate leafy stems the uppermost leaf traces sometimes take all remaining vascular tissue.

Aquilegia and *Scheuchzeria* represent the two general types of behavior of the tip of the vascular cylinder in flowers. In this important respect flowers are like other determinate stems.

There is a close resemblance of all organs to leaves in their anatomical relations with the axis: in the derivation, number, and course of the traces, and in the presence and character of the gaps. The flower stele resembles the stele of determinate stems generally.

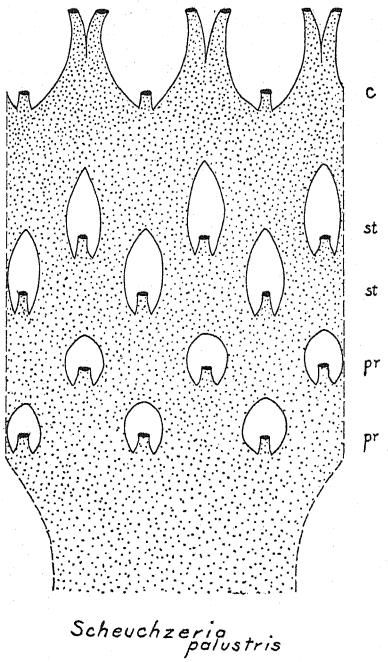


TEXT FIG. 3. *Scheuchzeria palustris.* Cross sections of the flower at successive levels: *a*, the pedicel; *b*, traces of the lower perianth whorl and their gaps; *c*, traces of the six perianth parts far out, three lower stamen traces out, three upper departing; *d*, the vascular cylinder unbroken above the gaps of the stamens; *e*, the dorsal carpel traces out, leaving the cylinder broken into three strands by their gaps; *f*, the carpel loculi showing, the receptacle bundles each giving rise to two ventral carpel traces; *g*, the carpels nearly free, the ventral traces half inverted; *h*, *i*, *j*, the carpels at successive levels, the ventrals are inverted.

Let us next consider the anatomy of the various floral organs individually.

Sepals. The sepals are, with very few exceptions, anatomically like the leaves of the plant in question. The traces are similar in origin and number. From the evidence of the vascular system, sepals are clearly, in nearly every case, morphologically bracts,—that is, they have been derived directly from leaves and are not sterile sporophylls.

Petals. The petals are in their vascular supply sometimes like leaves, but much more often like stamens. They may have one, three, or several traces; most commonly there is but one trace regardless of the size, shape,



TEXT FIG. 4. *Scheuchzeria palustris.* The vascular cylinder of the receptacle represented as split longitudinally and spread out, showing the position, number, and relations of the traces and gaps of the organs. The vascular cylinder does not extend beyond the carpel traces.

or persistence of the organ. Petals appear to be sometimes modified leaves, like the sepals, but in the great majority of families they are sterile stamens. However, since stamens are the homologues of leaves, it is not always possible to determine from anatomical evidence alone whether one-trace petals in certain families are modified stamens or whether they have come more directly from leaf-like structures.

Stamens. A single trace is usually found as the supply of stamens, regardless of the size, shape, character of base, etc. However, in a few families, such as the Magnoliaceae, Lauraceae, and Musaceae, stamens with three traces occur; and since from other evidence, these appear to be

fairly primitive groups, it seems highly probable that the single trace condition is one of reduction from three. That is to say, as the primitive stamen with three traces became more attenuate, its vascular supply was reduced to one bundle. Evidence for this cannot be considered here, however.

Carpels. Anatomically the carpel is the most complex of the floral organs; not, however, because it differs in its vascular supply, which is closely like that of the other organs, but because it is a folded structure, and because carpels are the uppermost (innermost) organs of the flower and hence are most affected by cohesion and adnation.

The carpel is commonly looked upon as a leaf-like organ folded upward, *i.e.* ventrally, with its margins more or less completely fused and bearing the ovules. This conception is borne out by the anatomy. The details of origin, number, and course of the bundles forming the vascular supply are exactly like those of leaves, and the upfolding and fusion are histologically evident.

The Angiosperm leaf is known to have 1, 3, 5, or many traces derived usually from the same number of gaps. These traces arise often wide apart, the laterals even on the side of the stem opposite to that where the midrib trace arises. The 3-trace condition, which is most common, appears to be primitive, the others being derivative conditions. The 1-trace condition seems to have resulted from the fusion of the lateral traces with the median trace, or perhaps in some cases from the loss of the lateral traces; the condition with more than three traces seems to have arisen by the multiplication of an original three.

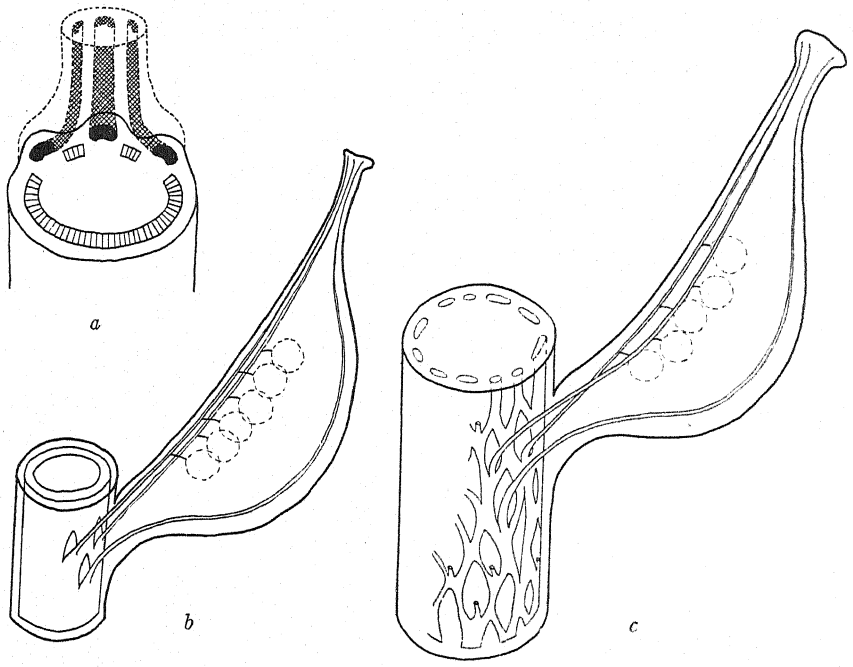
The carpel parallels the leaf closely in its vascular anatomy, and also in the evolutionary modification of its skeletal tissues. There are 1-, 3-, 5-, and several-trace carpels. The 3-trace condition here is also the common one, and is characteristic of the type of carpel which is generally accepted as primitive, the follicle. The evidence that the 1-trace carpel (nearly always an achene) has been derived by reduction from the 3-trace type is overwhelming. The 5-trace carpel is nearly as common as the 3-trace, and carpels with 7, 9, and more traces are increasingly less and less common.

The carpel and the stamen add to the evidence that the foliar appendage of the Angiosperms was primitively a 3-trace, palmately veined structure. The leaf itself suggests this, and the very conservative reproductive structures greatly strengthen the view.

Let us now consider typical carpels and their fundamental vascular supply. The simple carpel closely resembles a leaf in this respect. Text figure 5*a* shows the number and origin of the traces to a typical leaf: three traces, a median and two laterals from three separate gaps. The lateral traces arise at some distance from the median trace laterally, and slightly higher on the stem. The lateral traces often arise far around on the stem from the median trace, even on the opposite side, and especially is this the

case when there are 5, 7, or more traces. (The figure shows only a common 3-trace condition.)

Text figures 5*b* and 5*c* show the closely similar vascular supply of a carpel, three traces from three gaps,—in figure 5*b* from an unbroken vascular cylinder, in 5*c* from a dissected cylinder. The median trace departs first, the laterals higher up, as in a leaf. The median trace runs along the midrib of the carpel on its dorsal side, and hence is called the dorsal trace or dorsal bundle. The lateral traces run along the margins of the folded carpel, *i.e.* along its ventral side, and are, therefore, called the ventral or marginal traces, or bundles. These bundles become inverted before they enter the



TEXT FIG. 5. Diagrams showing similarity of leaf trace and carpel trace: *a*, origin of the traces of a typical leaf (modified, after Sinnott and Bailey); *b*, *c*, traces to a typical carpel, from an unbroken and from a dissected stele, respectively.

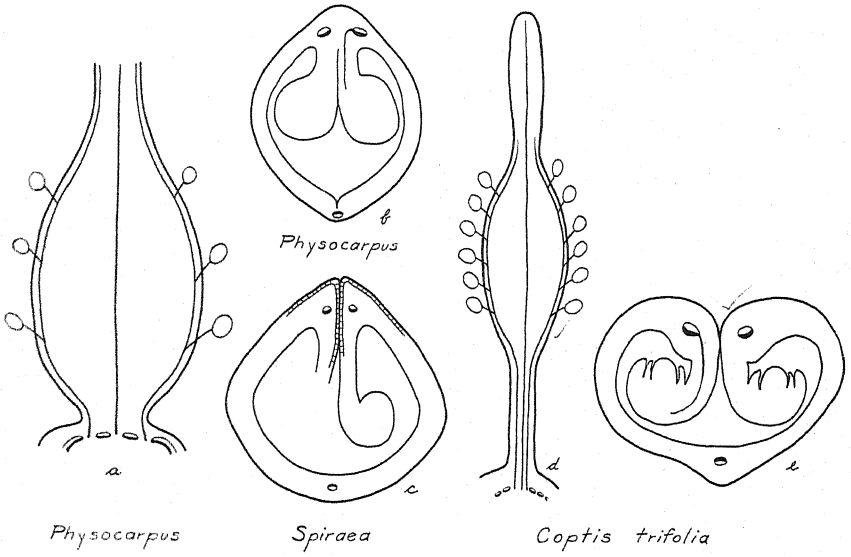
carpel (this not shown in figures), the phloem being on the ventral side in the carpel, whereas it is on the dorsal side in the midrib bundle. This important condition is readily understood when it is remembered that the carpel is leaf-like, with its margins folded upward. The upfolding inverts the marginal parts of the carpel and hence its marginal bundles. The ovule traces are derived from the ventral bundles.

Let us now look at the structure of follicles from various families which are considered to have at least fairly primitive flowers.

Spiraea and *Physocarpus* (Rosaceae, text fig. 6, *a*, *b*, *c*) are good ex-

amples. The cross sections of the ovaries show the upfolding and the inversion of the ventral bundles. In *Spiraea* the fusion of the margins is still so incomplete that the outer epidermal layers continue uninterruptedly through the region of fusion, giving further proof, if that were needed, that this ovary represents an upfolded leaf. In *Physocarpus* the fusion line, though still evident histologically, does not show the double epidermal layer. Text figure 6*a* shows the carpel split down the ventral edge and unfolded. The origin and course of the three traces is seen, also the attachment of the ovules.

Coptis (Ranunculaceae, text fig. 6*d*, *e*) likewise shows well the folded nature of the carpel and the inverted bundles. This follicle likewise has three traces, in spite of its much constricted base. The three traces are distinct, but derived all from one gap. They run close together, though entirely separate, through the stipitate base.

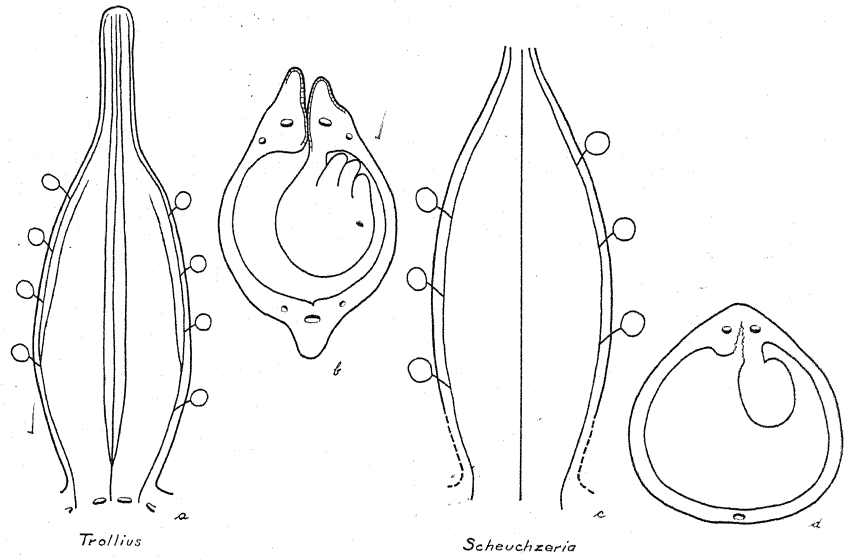


TEXT FIG. 6. Typical carpels: *Physocarpus opulifolius*, *a*, split ventrally and spread open, showing trace number and origin, and venation; *b*, cross section; *Spiraea salicifolia*, *c*, cross section; *Coptis trifolia*, *d*, *e*, as in *Physocarpus*.

Trollius (in the same family, text fig. 7*a*, *b*) shows the typical three traces from three gaps, and a very slight fusion of the margins, the epidermal layers being still present on the united margins. The midrib has two branches near the base, and each of the ventrals has one.

Branched dorsals occur also, for example, in the follicles of *Tetracentron* (Trochodendraceae, text fig. 8*c*) and of *Platanus* (Platanaceae, text fig. 8*a*). These are typical 3-trace carpels. In *Tetracentron* the carpels are fused at the base. The pistil of *Platanus* is really an achene with the structure and

vascular supply of a follicle. Like a primitive follicle it is not wholly closed, the loculus being freely open to the outside for some distance. There are often two ovules, and when but one, the vestigial trace to the second is present.



TEXT FIG. 7. Typical carpels: *Trollius laxus*, *a*, *b*, as in *Physocarpus*, fig. 5; *Scheuchzeria palustris*, *c*, *d*, also as in *Physocarpus*.

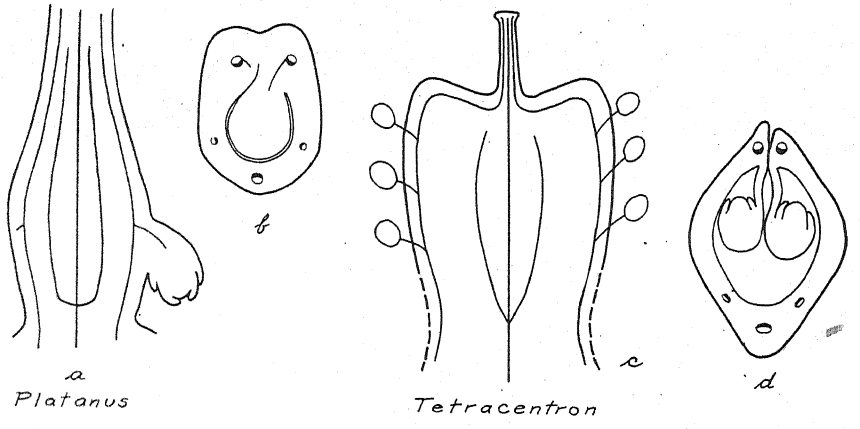
In all these carpels there is characteristically a pair of inverted ventral bundles from which the ovule traces are derived; the bundles are sometimes very close together, again farther apart, dependent upon the character of the carpel margins.

Scheuchzeria (Juncaginaceae, text fig. 7*c*, *d*) provides a typical follicle from the Monocotyledons. (The follicles cohere for a short distance at the base, see fig. 3*f*, *g*, *h*, *i*). Here again are the usual (typical) three traces, arising independently.

Let us now turn to follicles with slightly greater vascular supply. *Banksia* (Proteaceae, text fig. 9*a*, *b*) and *Sedum* (Crassulaceae, text fig. 9*c*, *d*) serve as examples. There are here five bundles in the carpel, and five traces derived independently from the stele of the receptacle. The two extra traces lie between the dorsal and the ventrals, one on either side. These traces, which may be called "median laterals," deserve particular notice, as they are of much importance from the standpoint of the polymorphism theory. Note that these carpels are, like some of the others discussed, primitive in that their margins are only slightly fused, the epidermal layers of the united edges being still distinct. (This condition is regarded under polymorphism, teleologically, as a provision for dehiscence,

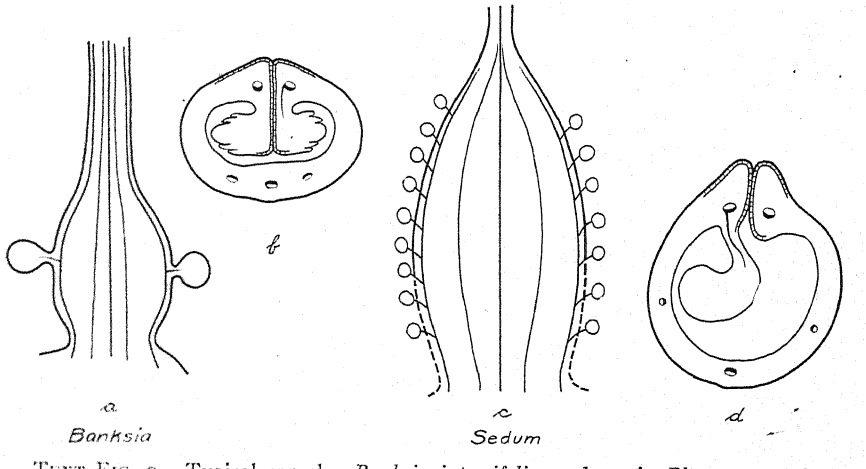
—the solid, or semi-solid, ventral carpel being prepared with a double bundle for ready splitting. It is worthy of note here that often, even when epidermal layers occur in this 'provision,' dehiscence occurs elsewhere, or still more often, does not occur at all.)

Types of carpels with more than five traces are not considered at this point as they will be discussed later.



TEXT FIG. 8. Typical carpels: *Platanus occidentalis*, *a*, *b*, as in *Physocarpus; Tetracentron*, *c*, *d*, also as in *Physocarpus*.

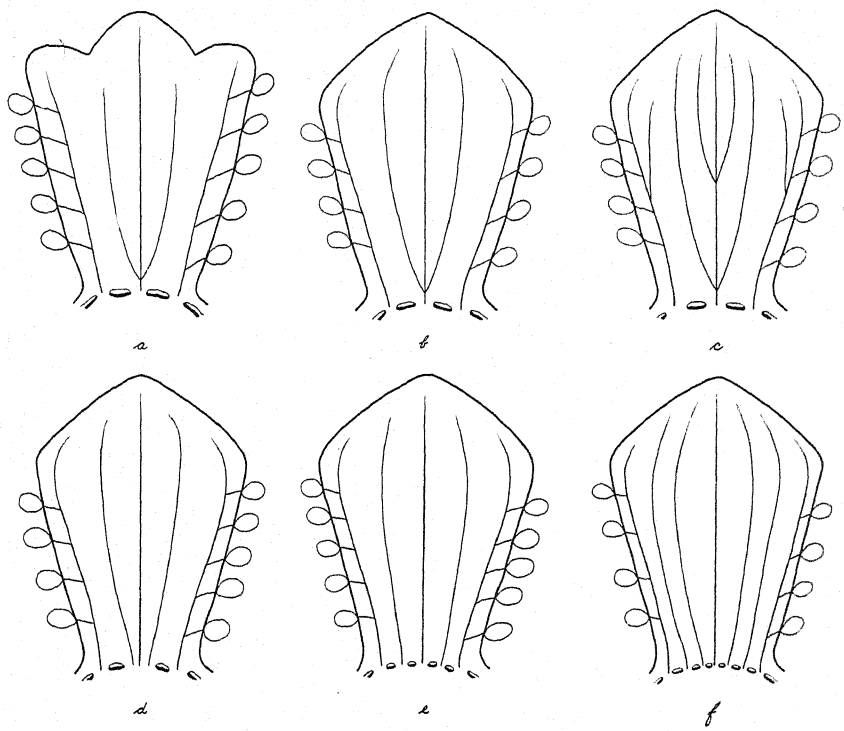
The intensive study of the carpel throughout the Angiosperms makes it clear that the primitive carpel was a palmately veined structure with three strong traces (perhaps with two or more additional, weaker traces) derived from as many gaps. The lateral veins were strongly developed and, like



TEXT FIG. 9. Typical carpels: *Banksia integrifolia*, *a*, *b*, as in *Physocarpus*, fig. 5; *Sedum ternatum*, *c*, *d*, also as in *Physocarpus*.

the midrib, extended to the distal part of the lamina. The ovules were several to many and marginal only, deriving their traces from the ventral bundles.

Text figure 10 shows diagrammatically what the primitive carpel seems to the writer to have been like. The palmate venation and the three traces from three gaps are the outstanding features. It seems almost certain also that this primitive carpel was not only palmately veined, but palmately lobed as well, that is, the 3-lobed type (fig. 10*a*) is much more probable



TEXT FIG. 10. Diagrams of theoretical primitive carpels, showing palmate venation and three or more traces from as many gaps.

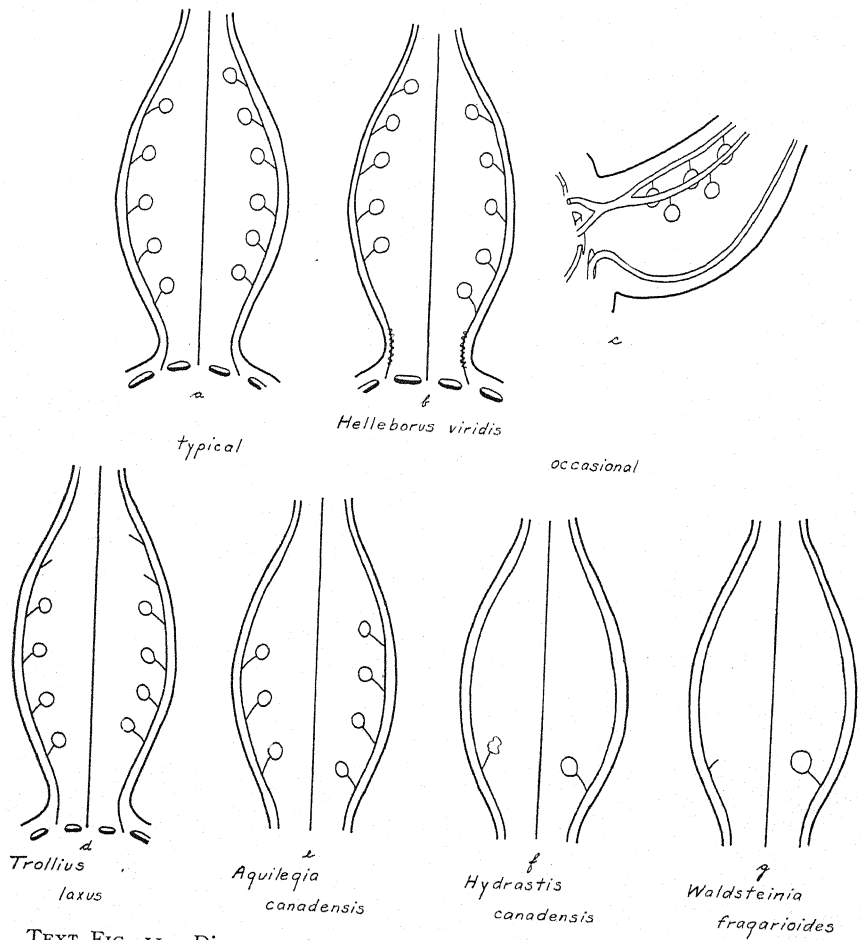
than an unlobed type (fig. 10*b*, *c*). The evidence for this is (1) the fact that throughout Angiosperms the ventral bundles are strong and run (except in obviously specialized forms) into the ventral portion of the stigma; (2) the lobing of the stigma often suggests that this part of the carpel is made up of two or of three parts (one and a pair), and the dorsal bundle or its branches supply one part, the ventrals the other one or two. The presence of lobes in the primitive carpel seems certain, therefore, but there is little evidence as to the prominence of these lobes.

Branches of the dorsal bundle (text fig. 10*a*, *b*, *c*) are apparently charac-

teristic, and their presence is of importance morphologically, because of their relation to the polymorphism theory.

Text figures 10*b*, *c*, *d*, *e*, *f* represent other carpel types, all found not infrequently among fairly primitive Angiosperms. These may also be primitive types, but seem much more likely to represent derivations from the type in fig. 10*a* by increase in the vascular supply, chiefly through branching of the midrib, and by a working back of this branching tendency to the stele. The 5-, 7-, and several-trace types may in this way have been derived from the 3-trace type.

Though it is only probable that the carpel with more than three traces



TEXT FIG. 11. Diagrams showing vascular supply of follicles. *Helleborus viridis: a*, typical carpel; *b*, *c*, occasional carpel, with ventrals fused for a short distance. *Trollius*, the ovules reduced in number, traces to the lost ovules persisting; *Aquilegia*, upper ovules lost, no traces remain; *Hydrastis*, all ovules but lowest two lost, one of these abortive; *Waldsteinia*, all ovules but one lost, trace to one other persisting.

is a somewhat specialized one, and derived from the 3-trace type, there is everywhere abundant proof that the 1-trace type has been derived from the 3-trace one. There is no evidence whatsoever of primitive 1-trace carpels; the 1-trace condition is certainly due to reduction.

Let us consider briefly some of the evidence for this. The taxonomist and the morphologist have long said that the (true) achene is a specialized carpel representing a follicle reduced in size and in number of ovules. The morphological evidence for this opinion is strongly reinforced by the anatomical facts. The Ranunculaceae and Rosaceae provide excellent examples of the derivation of the achene as shown by anatomy.

Helleborus (text fig. 11*a*) possesses a typical, multiovulate, 3-trace follicle. *Trollius* (fig. 11*d*) has a similar follicle, but the uppermost ovules have been lost, and proof of this exists in the presence of vestigial traces in the position where these ovules formerly existed. In *Aquilegia* (fig. 11*e*) the upper ovules have likewise disappeared, leaving the top of the follicle empty, and even their traces have been lost also. In *Hydrastis* (fig. 11*f*) all but the two lowermost ovules have been lost, and one of these two is vestigial. In *Waldsteinia* (Rosaceae, fig. 11*g*) only one ovule remains, though the trace to the lowermost one on the other margin is still present. All of these carpels have three traces and the achene of *Waldsteinia* is merely a small follicle. The reduction is essentially that of ovules only.

These genera also show the first stages of the reduction in the vascular system. Reduction in size of the ovary brings the traces closer together at the base, and this approximation leads to fusion. Occasional carpels in *Helleborus*, *Aquilegia*, *Hydrastis*, and other Ranunculaceous genera have the structure shown in text figures 11*b*, *c;* the two ventrals are fused for a short distance just beyond their origin—in the region where they come most close together.

When reduction in the ovary is greater, this fusion becomes more extensive, reaching for considerable distances along the ventrals, or throughout their length, and even including the dorsal bundle from its origin to the base of the loculus.

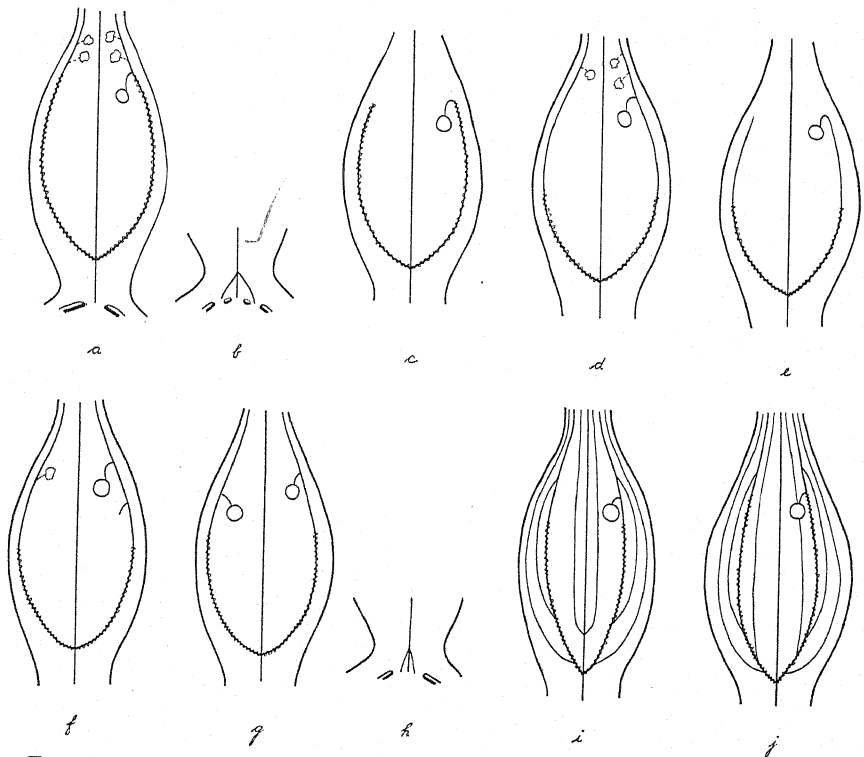
This more extensive fusion of the skeleton is particularly clear in those Ranunculaceae and Rosaceae where stages of achene formation occur with one of the uppermost ovules persisting. The following types illustrate this.

Anemone canadensis (text fig. 12*a*, *b*) shows commonly but one trace to the carpel; but frequently three traces from three gaps occur, and the three traces fuse into one as they enter the base of the pistil, the triple nature of the fusion bundle being sometimes evident as far as the point of splitting above. The ventrals are fused as far as the normal ovule near the top of the loculus. Beyond this point the two ventrals are free into the stigma. Two to four vestigial ovules occur above the normal one. It is obvious that the one trace of this achene represents a reduction condition, the three traces being fused in their origin and as far as the base of the

loculus. Beyond this point the ventrals are still fused for nearly the length of the loculus.

A. virginiana and *Clematis virginiana* (text fig. 12*c*) show a still more reduced condition, the triple trace being rarely found, the vestigial ovules lacking, and the ventrals not continued beyond the ovule.

Turning to the Rosaceae, similar conditions are found. *Potentilla recta* (text fig. 12*d*) resembles *Anemone canadensis*, and *P. canadensis* (fig. 12*e*) is similar to *Anemone virginiana* except that in both cases the ventrals are



TEXT FIG. 12. Diagrams showing the vascular supply of achenes with an upper ovule persisting. Stages in the reduction of the skeleton and the fusion of its parts are shown. The wavy lines indicate fusion bundles, which must be split if the carpel is to be opened ventrally and spread out as shown. *Anemone canadensis: a*, the typical carpel, with one trace; *b*, the occasional carpel, with three traces; the ventrals are fused as far as the normal ovule. *A. virginiana* and *Clematis virginiana: c*, the ventrals are fused as far as the ovule, and do not extend beyond. *Potentilla recta: d*, the ventrals are fused only part way to the ovule, and extend beyond. *P. canadensis: e*, the ventrals are fused only part way to the ovule and do not extend beyond its level of attachment. *Dalibarda repens: f*, the ventrals extend, fused, part way to the ovule, and beyond into the style; vestigial ovule traces are present. *Rubus* spp.: *g*, the typical carpel, with one trace, the ventrals fused part way to the ovules; *h*, the frequently found carpel, with three traces. *Anemonella thalictroides, i*, and *Thalictrum polygamum, j;* vascular supply much stronger, the ventrals fused as far as the ovule in one, and nearly as far in the other.

fused only part way to the ovule. *Rubus* (fig. 12*g*) and *Dalibarda* (fig. 12*f*) show further stages in the derivation of the achene from the follicle. In the former there are two normal ovules, in the latter, there is one normal ovule, one abortive one, and a trace to the position of a third. In both genera the ventrals are fused only part way to the ovule. Some species of *Rubus*, notably *R. allegheniensis* and *R. flagellaris*, show frequently the triple origin of the carpel trace (fig. 12*h*), though the three bundles in this case always arise from one gap, a more advanced condition.

This condition of the clearly triple origin of the apparently single trace, which is found in many achene genera, together with the otherwise conclusive proof that the achene is a reduced follicle (and follicles have been shown to have three traces), demonstrates the fact that the single-trace carpel is the result of reduction from the 3-trace carpel. In the study of many achene-bearing families every one presented evidence that the carpel (if it had but one trace) had been derived from a three-trace type.

The similar achenes of *Anemonella* and *Thalictrum* (fig. 12*i*, *j*) show in their much greater vascular supply further evidence of the palmate nature of the venation.

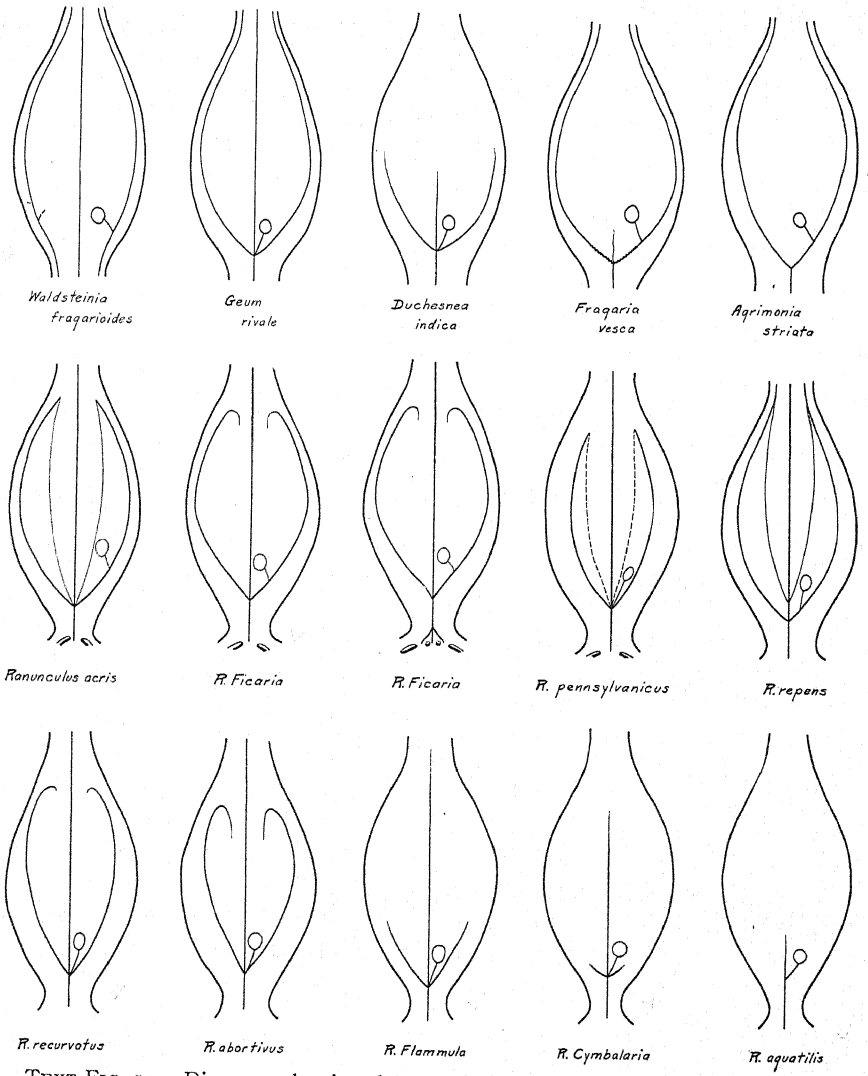
Achenes of a more extreme type in the same families present the story of further reduction of the vascular skeleton. It is apparent that the greater reduction is due in large part to the fact that the persisting ovule is one of the lowermost, rather than uppermost as in the forms just discussed. With the ovule in this position the vascular tissue tends to disappear in the distal parts.

A series from the Rosaceae and one from the genus *Ranunculus* are shown in text figure 13. In both, the vascular tissues are seen to be reduced from the style toward the base. In the former the dorsal bundle is reduced more rapidly; in the latter the ventrals. In both groups the ventrals, though running close together, remain unfused.

The Rosaceous genera show a reduction of the dorsal bundle to its complete loss (in its free part) in *Agrimonia*. (The ventrals are also greatly reduced in *Duchesnea*.) The lateral position of the ovule is noticeable in *Fragaria* and *Agrimonia;* its pseudomedian position in *Geum* and *Duchesnea* is due to the fact that the dorsal and ventral bundles are fused as far as the point of departure of the ovule trace.

The genus *Ranunculus* reveals within itself the history of reduction where the ventrals are lost and the dorsal persists. Various species, such as *R. acris*, *R. repens*, *R. Ficaria*, show well-developed vascular supply (some species with the branches of the dorsal present), and with the fusion of the three main veins not continued as far as the ovule, so that the latter is obviously lateral, its primitive and true position. Other species, such as *R. pennsylvanicus*, *R. recurvatus*, and *R. abortivus* show fusion nearly or quite to the ovule, so that the ovule seems to be attached to the dorsal bundle at the point where the ventrals depart, and hence to be basal rather

than lateral. *R. Flammula* and *R. Cymbalaria* show the same condition, with the ventrals very much reduced, in the latter species to mere stubs. The condition in these species explains the extreme reduction found in *R. aquatilis* where the ovule seems to be borne on the dorsal bundle—a condition never actually present in Angiosperms (though sometimes apparently existing).



TEXT FIG. 13. Diagrams showing the vascular supply of achenes with a lower ovule persisting. The vascular supply is reduced from the style toward the base, the dorsal bundle more rapidly in the Rosaceous forms, and the ventral bundles in the Ranunculaceous forms. Both groups show how an ovule may seem to be attached to a dorsal bundle, though actually on a ventral.

The ventrals in this and other closely related species have entirely disappeared (except in so far as they exist in fusion with the dorsal). The dorsal extends only a short distance beyond the point where the ovule trace is given off. No greater reduction than this has been found in this family, but in other groups the ovule appears to be terminal upon the single strand which enters the carpel. This "basal" position is clearly a derived or false one, and the single bundle is not a mere ovule supply, but the remnants of the supply to the carpel, terminating in the ovule trace.

Even so reduced an achene as that of *Ranunculus Ficaria* shows proof of its origin from a 3-trace carpel, for carpels are found occasionally or frequently with three traces from three gaps (text fig. 13).

The story of vascular reduction in the achene is as complete as could be desired. It would alone supply proof that the achene is a reduced follicle. Taken together with the morphological evidence it cannot be questioned that the one trace of the achene is reduced from three.

And with very few exceptions all one-trace, one-carpel pistils are achenes. These exceptions, for example, *Zanthorhiza* (Ranunculaceae) and *Zanthoxylum* (Rutaceae), are reduced follicles usually with two ovules. That one-trace carpels exist in Angiosperms only under reduction is obvious.

Cohesion

In the specialization of the flower fusion of parts and reduction have been the outstanding changes. All stages of cohesion, adnation, and reduction may be found in many groups.

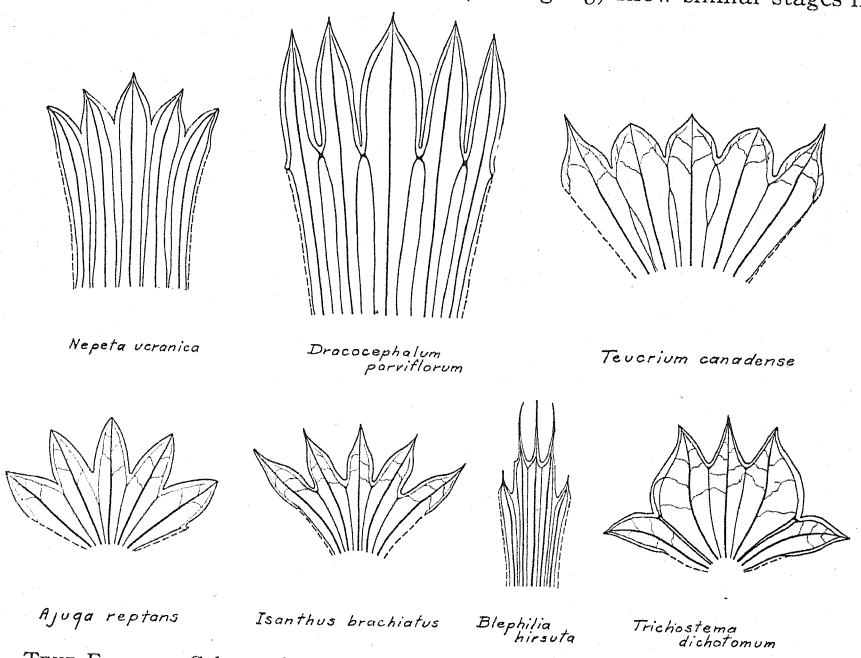
In the history of fusion, the organs become united externally at first, and the internal structure is not modified. Ultimately the fusion becomes most intimate and involves the vascular tissues whenever these lie sufficiently close to one another.

The effect of cohesion upon the vascular skeleton can be simply shown by the following examples.

The gamosepalous calyces of the Labiatae, the Caryophyllaceae, and other families present in different genera, and even in different species of the same genus, various degrees of intimacy of fusion. Further, in the Labiatae (text fig. 14), zygomorphy has brought some sepals more close together than others, with more intimate fusion in the case of the former. The sepal of this family has three traces and three main veins. *Nepeta ucranica* shows simple gamosepaly where the vascular bundles are not affected. In *Dracocephalum parviflorum* the lateral veins of each sepal are fused with those of the adjacent sepal just below the sinuses. In *Teucrium canadense* the pairs of laterals are fused for considerable distances, in some cases throughout. *Ajuga reptans* and *Isanthus brachiatus* show all pairs united throughout. The more zygomorphic calyx of *Blephilia hirsuta* shows two pairs united and three free; that of *Trichostema dichotomum* three pairs united and two pairs free. (The intimacy of the fusion and the

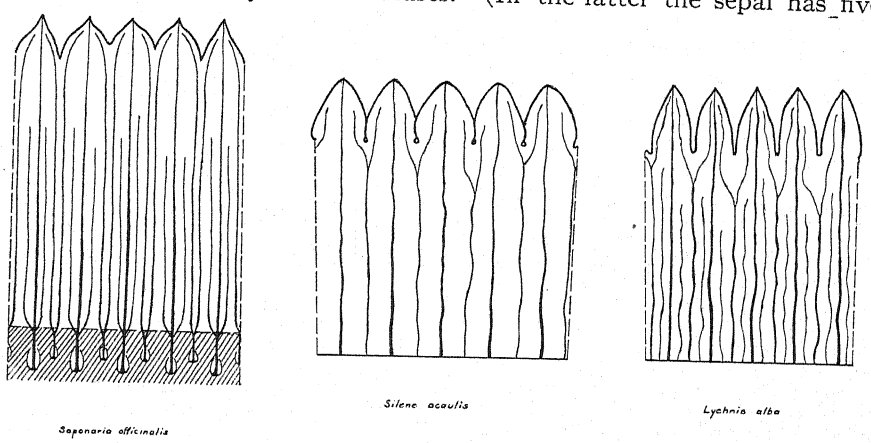
extent of the zygomorphy control the valuable taxonomic character of number of ribs in the calyx.)

The calyces of the Caryophyllaceae (text fig. 15) show similar stages in



TEXT FIG. 14. Calyces of the Labiatae, showing the tendency of the lateral bundles to fuse under cohesion.

fusion, though without the effects of zygomorphism. In *Saponaria officinalis* the lateral bundles are free (except in their origin); in *Silene acaulis* they are fused nearly to the sinuses. (In the latter the sepal has five



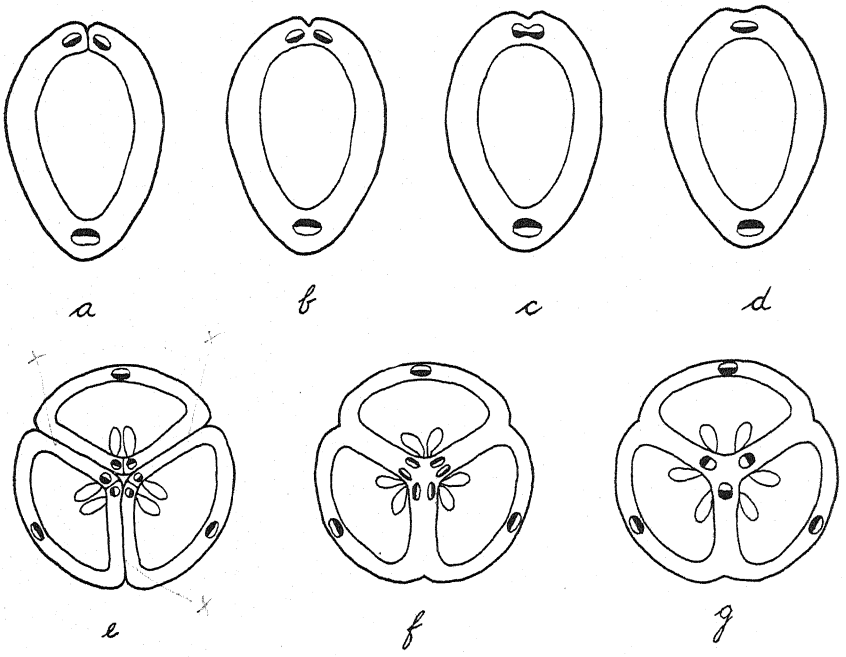
TEXT FIG. 15. Calyces of the Caryophyllaceae, showing the fusion of lateral bundles under cohesion, and the common origin of the lateral bundles of adjacent sepals in *Saponaria.*

traces.) Note the origin in *Saponaria* of the lateral traces of two sepals as one bundle. The polymorphism theory attaches much weight to the fact that traces to two distinct organs cannot arise together, *i.e.*, as one bundle, yet this is a condition commonly found under both cohesion and adnation.

The corolla of the Compositae shows similar stages in the fusion of the lateral veins of the petals.

In the ovary the effects of cohesion are similar: vascular bundles which lie close to one another become fused wherever the union of carpels has become intimate.

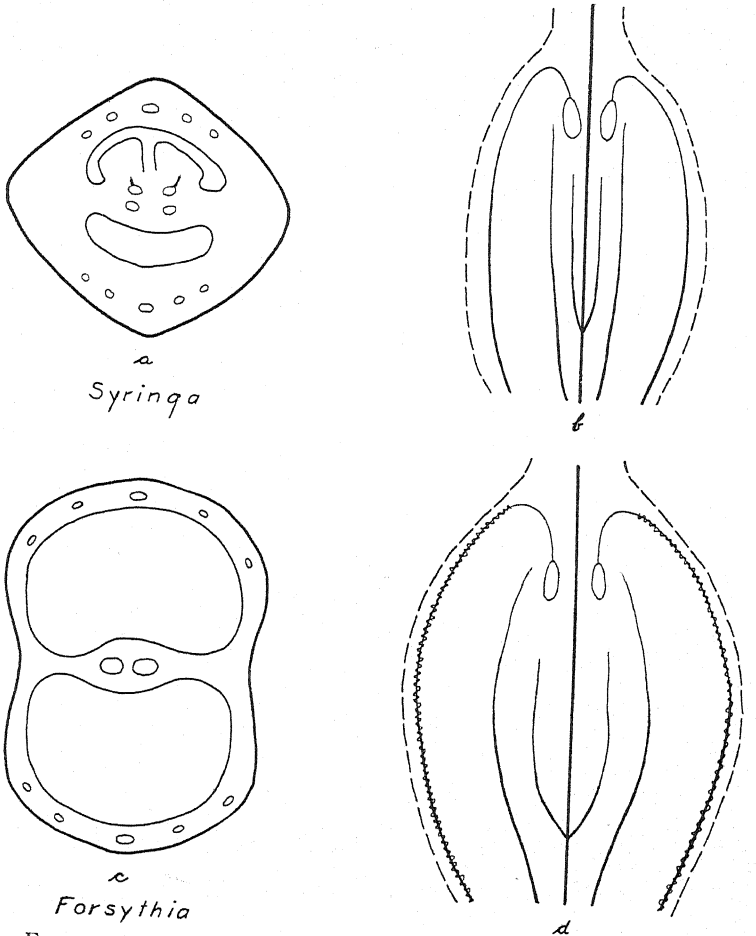
The folding of the carpel forms the simplest type of cohesion here. This



TEXT FIG. 16. Diagrammatic cross sections of typical ovaries showing the effects of cohesion upon the vascular skeleton: *a–d*, showing stages in the fusion of the ventral bundles of a follicle; *e–g*, showing the fusion of carpels and the union of ventrals of adjacent carpels.

brings the two ventrals close together (text fig. 16*a*), forming the characteristic pair of bundles on the ventral side of follicles (text figs. 1*j*, 3*j*; 6*b*, *c*, *e*; 7*b*, *d*; 8*b*, *d*; 9*b*, *d*). In closer fusion the epidermal layers disappear (fig. 16*b*) and the two bundles become one double bundle (fig. 16*c*), or a single simple strand (fig. 16*d*). The union of these two ventral bundles may be for a short distance only, or throughout their extent, even back to the point of origin, where the two strands may arise as one, a frequent condition in many families.

Syncarpy, the cohesion of separate carpels (fig. 16*e*) involves similar changes. The lines separating the carpels and the margins disappear (fig. 16*f*). The ventrals—inverted, with the xylem toward the outside of the ovary—form a ring in the centre. They lie, usually very definitely, in pairs,—a pair consisting of the ventrals of the same carpel, or, more often, of bundles from each of two adjacent carpels. The members of a pair tend to fuse, and all degrees of fusion are found,—approximation only, double



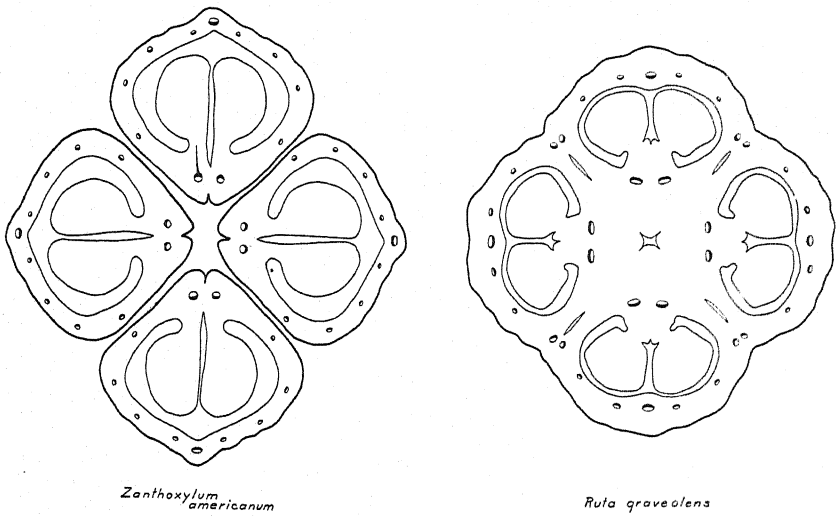
TEXT FIG. 17. Vascular supply of the ovary of *Syringa* and *Forsythia* to show the fusion of the ventrals in pairs with those of the opposite carpel: *a*, *c*, cross sections of the ovary; *b*, *d*, vascular supply of a single carpel.

bundles, single bundles. Whether the fusing ventrals belong to the same carpel or to adjacent carpels is determined by the character of the carpel margin, by the position of the ventrals in the carpel margin, and by the

extent of inrolling of the margin in the folding of the carpel. Text figure 16*g* shows the more common condition where the bundles of adjacent carpels have fused. The important point is that the ventrals may fuse completely (just as do the laterals of sepals and petals under cohesion), and that the fusion may extend even to the point of origin of the traces in the receptacle, (again just as does fusion in these other organs).

Syringa and *Forsythia* (Oleaceae, text fig. 17) serve as an example of this fusion of ventrals. In *Syringa* the ventrals are free; in *Forsythia* they are fused in pairs, one from each carpel in each pair. (These ovaries provide examples of five-trace carpels.)

Where there are branches of the dorsal bundle or where there are five

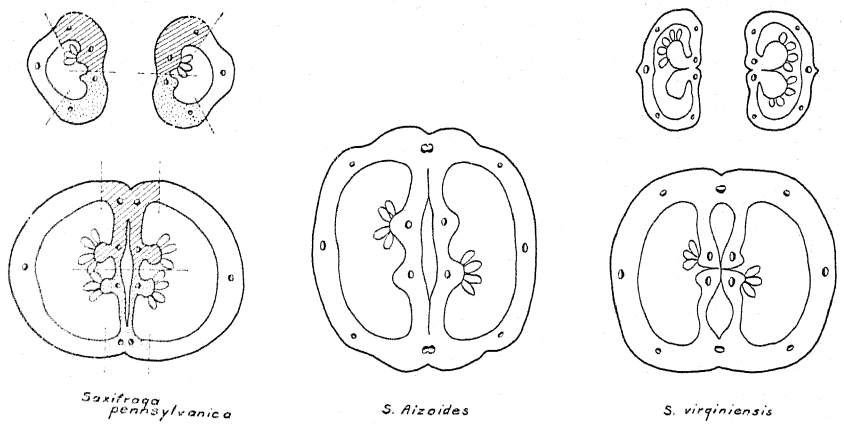


TEXT FIG. 18. Diagrammatic cross sections of the gynoecium of apocarpous and syncarpous types in the Rutaceae to show the approximation in pairs of the lateral bundles of the carpels.

or more traces, these additional bundles may also be brought together in pairs by the union of the carpels. This is a most important point because of its bearing on the polymorphism theory. *Zanthoxylum* and *Ruta* (Rutaceae), apocarpous and syncarpous forms, respectively, show this point (text fig. 18). Note that in *Zanthoxylum* the wholly free carpels are approximated and the inner lateral bundles form pairs, though in separate carpels. In *Ruta* the same bundles form close pairs, not separated even by epidermal lines. Note just inward from the pairs of bundles the remnants of the intercarpellary spaces, the equivalent of the septal glands of the Liliaceae (text fig. 23*a*, *b*). There is a striking resemblance to the condition found in the Liliaceae where the polymorphism theory claims the cavity to represent the vestiges of the loculus of a solid carpel and the pair of bundles

to be the midrib of this carpel. In this family it is obvious what these structures are.

The genus *Saxifraga* (text fig. 19) provides us with similar stages in fusion. The carpels are fused only in their basal parts, and the ventrals, because of the shape of the carpels, are not fused. The sides of the carpels are, however, brought closely together, and the median laterals show all stages of fusion in the many species. *S. pennsylvanica*, for example, has



TEXT FIG. 19. Diagrammatic cross sections of the ovaries of species of *Saxifraga* to show stages in the fusion of the lateral bundles of different carpels,—separate in *S. pennsylvanica*, slightly fused in *S. Aizoides*, and completely fused in *S. virginiensis*. The shading in the figures of *S. pennsylvanica* indicates the limits of the "solid carpels" of polymorphism.

these bundles widely separated; *S. Aizoides* has them slightly fused; and *S. virginiensis* has them completely fused.

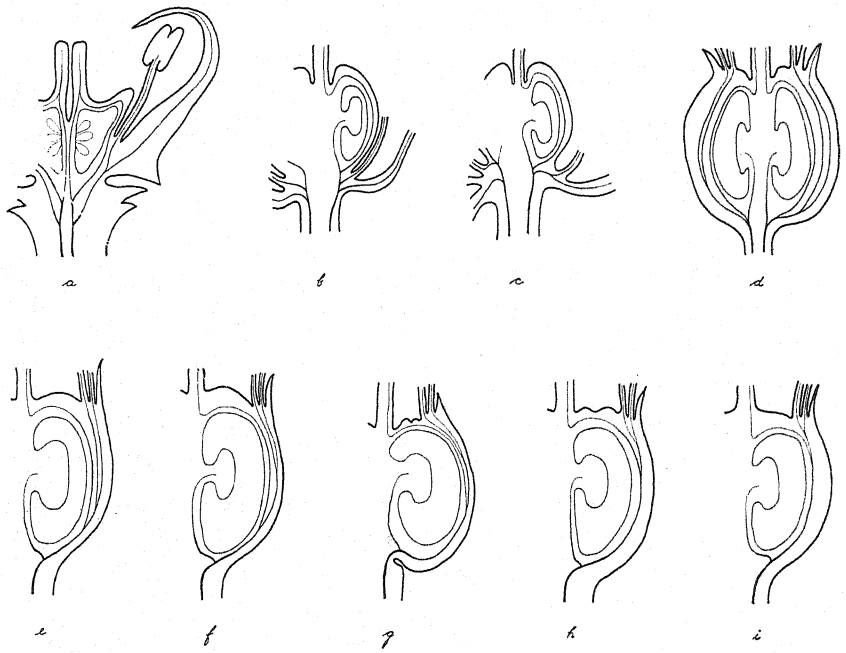
ADNATION

The fusion of members of one whorl with those of another affects the vascular skeleton in the same manner as does cohesion. Whenever bundles lie close to one another radially or tangentially they may become fused. In many cases there is abundant ontogenetic and histological evidence that such fusion of vascular tissues has occurred; in other cases the organs are congenitally concrescent, and only the evidence of comparative study demonstrates the underlying morphological situation.

It is not possible within the length of this paper to discuss fusion under adnation to the same extent as fusion under cohesion. It may be said, however, that fusion occurs as readily between the vascular bundles of organs of different whorls as between those of the same whorl, and that closely similar conditions obtain as to stages and proof of the fusion. A few examples may be given.

Simple cases are abundant and very clear,—for example, the epipetalous stamen. The traces of a petal and of the stamen borne upon it may be separate throughout, or may be fused for any distance. Often the traces arise as one from the receptacular stele.

Tetracentron (text fig. 20*a*) shows the origin of a stamen and a perianth trace as one bundle. It shows, further, the adnation of stamen and perianth



TEXT FIG. 20. Diagrams to show stages in fusion of the vascular skeleton under adnation: *a*, *Tetracentron*, the sepal and stamen adnate to the ovary wall, the sepal and stamen traces fused, the carpel trace free; *b*, *Pyrola secunda*, a typical superior ovary type with no fusion of organs; *c*, *Andromeda glaucophylla*, with stamen base adnate to corolla, and the traces of these two organs fused nearly to the base of the stamen; *d*, *Gaylussacia frondosa*, the stamens, corolla, and calyx adnate to the ovary, but the vascular supplies of these parts largely free, fused only at the extreme base of the ovary; *e–i*, species of *Vaccinium*, showing progressively greater amounts of fusion of stamen and petal bundles and of these with the dorsal carpel bundle—(*e*, *V. vacillans; f*, *V. corymbosum; g*, *V. pennsylvanicum; h*, *V. canadense; i*, *V. macrocarpon*).

to the ovary. The fusion of these outer whorls to the ovary has not as yet become so intimate that the traces of the outer members are fused to the dorsal traces of the carpels.

The inferior ovary represents adnation in its extreme form. Comparative studies, made with the understanding that fusion of organs ultimately brings about fusion of the skeletal tissues also, demonstrate that the inferior ovary in nearly all—perhaps in all—families has resulted from the

adnation of the outer floral whorls to the carpels. Histological evidence of this is available also, but abundant proof is supplied by the course of the bundles, and the manner of their forking or splitting. This much discussed subject requires long consideration and can be gone into now only briefly.

The Ericaceae, with both superior and inferior ovaries, provide an excellent example of the proof that the inferior ovary is the result of adnation of an extreme type. *Pyrola secunda* (text fig. 20*b*) and *Andromeda glaucophylla* (text fig. 20*c*) serve as superior ovary types. In the former there is no adnation fusion whatsoever; the traces to all the organs depart from the stele individually, and well above one another as normally in a hypogynous flower. In the latter conditions are similar except that the petal and opposite stamen trace are fused for a short distance. *Gaylussacia frondosa* (text fig. 20*d*) and species of *Vaccinium* (text figs. 20, *e–i*) serve as inferior ovary types. *Gaylussacia* shows a vascular skeleton hardly different from that of *Andromeda*, a superior-ovary type. The outer whorls are fused to the ovary, but union of skeletal tissues has occurred to a slight degree only. The petal and opposite stamen bundles and the sepal and opposite stamen bundles are fused to about the same extent as are the petal and stamen bundles in *Andromeda*. In the radius of the sepal midrib the dorsal carpel bundle is fused for a very short distance with the united sepal-stamen bundle. In the radius of the petal the dorsal carpel bundle is free. The species of *Vaccinium* illustrated present a series showing progressively greater and greater amounts of fusion of the stamen and petal bundles, and likewise of this fusion bundle with the dorsal carpellary bundle. The points of separation in both cases are successively higher and higher in the ovary wall. In *V. macrocarpon* (text fig. 20*i*) the petal and stamen trace are fused to the free bases of those organs; and their fusion bundle is united with the dorsal carpellary bundle almost to the top of the loculus. In the first case fusion has extended as far as possible (unless the stamen were epipetalous); in the second case it is possible for fusion to take place as far as the base of the petal and sepal. Species of this genus do not show this extreme union, but this is a common condition in epigynous flowers. It is important to note that when the various bundles become free, the separation is often by a *tangential* split, and not by a radial division, as would be the case if these bundles were departing from a stele.

The Theory of Carpel Polymorphism

When the principles of the anatomy of the flower are understood—principles which are the same as those which govern the structure of stems and their appendages generally,—it is apparent that the anatomical structure of the flower bears out as a whole in a remarkable manner the usual interpretations of its structure made by taxonomy and morphology. It is only in unusual cases that existing opinions appear to be in error. This statement conflicts seriously with the claims of the recently proposed theory

of carpel polymorphism, but this new theory is based upon wholly unsound interpretations of anatomical structure.

Carpel specialization, reduction, and loss have indeed occurred freely, and there is much anatomical evidence of such changes. But that carpel modification along the lines proposed under the polymorphism theory has occurred is not in any way borne out by vascular structure.

The theory of polymorphism has gone far astray in its interpretations, and therefore also in its amazing conclusions, for the following reasons: (1) It fails to take into account the known anatomy of stems and leaves, —overlooking the fact that a flower is a "leafy" stem; (2) It neglects the determinate aspect of the flower stem and the consequent modification of the tip of the vascular cylinder, and therefore interprets bundles of the stele as traces of carpels; (3) It fails to take account of the effect of cohesion upon the vascular skeleton; (4) Its anatomical basis of interpretation is extraordinarily inconsistent; (5) It emphasizes the value of vascular tissues (even secondary venation) in determining fundamental morphology, yet in practice overlooks, neglects, or "discards" much such tissue; (6) It claims to explain gynoecium structure in a simple way and to remove all the difficulties of the older interpretation; yet its interpretations are as complex as, and often far more complex than, those of the accepted view and involve inconsistent and fantastic explanations; (7) It has taken the structural condition in a highly specialized family, and, mistaking analogous for homologous structure, has read into simpler forms the complex conditions of the higher. The difficulties in this have been extraordinary as evidenced by the tortuous methods of explanation, and by the inconsistencies and anomalies of interpretation.

Let us consider some of these points briefly.

1. The theory of polymorphism completely ignores our knowledge of the anatomy of appendages, overlooking especially the nodal structure of stems. The flower is a specialized stem and all the floral organs, from the best of evidence, are the homologues of leaves.

If stem structure had been at all considered in the development of this theory, such statements—of fundamental importance to the theory's existence—as the following could not have been made.

(a) *Traces to one organ cannot arise on opposite sides of the axis.* For example, the statement is made that the dimerous nature of the solitary ovary of *Poterium* "would seem to be established by the fact of the utilization . . . of two separate vascular cords *situated on opposite sides of the centre.*" If this curious basis of anatomical interpretation is accepted, the leaves of very many vascular plants are "dimerous" in nature!

(b) *In the gynoecium every trace* (with very rare exceptions) *determines a carpel.* A carpel is the homologue of a leaf, and the Angiosperm leaf has 1, 3, 5, 7, and many traces. The conservative carpel surely cannot be looked upon as always so unleaflike as not to show the fundamental leaf-

trace situation. Even the more specialized stamen still shows three traces in some cases.

(c) *Traces to two organs cannot arise as one.* This condition occurs freely under cohesion, as has been shown. Lateral traces of bracts, sepals, and petals, as well as those of carpels arise commonly fused with those of adjacent members in coherent whorls. Further, the expositions of polymorphism frequently mention the common origin of traces to different organs under adnation, a more extreme condition, surely.

(d) *An organ receiving all the "residual" vascular tissue* (except that which is "discarded"!) *cannot be lateral.* But this condition is found in the case of the uppermost leaf of herbaceous stems which are not terminated by an inflorescence, and in indeterminate woody twigs. Further, in the latter case the leaf is sometimes pseudo-terminal, as in the Leguminosae, thus closely resembling the pseudo-terminal carpel in the same family.

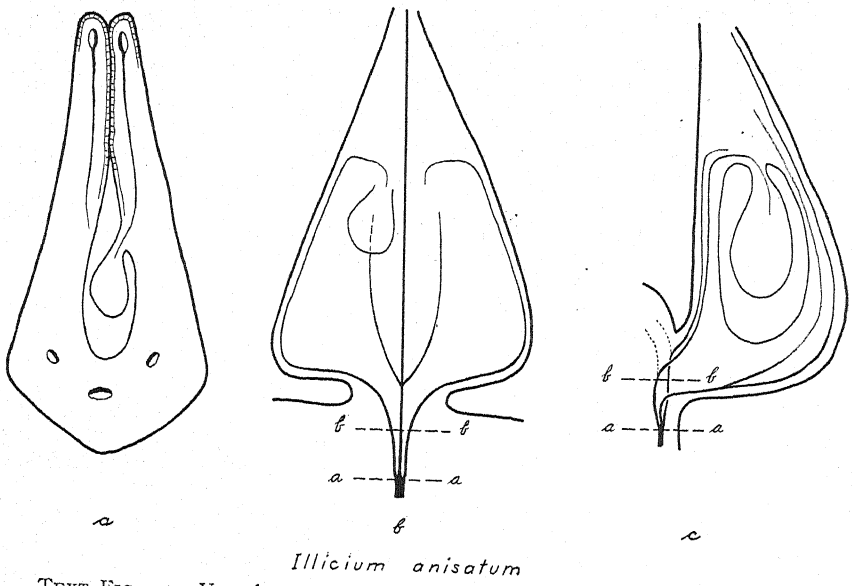
2. One of the two greatest errors into which the polymorphism theory has fallen is the interpretation of bundles of the receptacle as carpel traces. The basis adopted under polymorphism that the point of departure of a bundle from the stele determines a trace is certainly sound. The difficulty lies in determining in flowers what is the actual point of departure of the trace in question. When the receptacular stele extends beyond the uppermost traces there can really be no difficulty, provided the stelar tissues are recognized as such.

The situation may be understood by reference again to *Aquilegia* (text fig. 2*b*). There seems to be no question here as to how many traces there are to the carpel, nor as to the relation of the traces to the vascular cylinder. Yet if all vascular tissue above the level of departure of the ventral traces be considered of no consequence—mere discarded, superfluous cells (as such tissue is treated under polymorphism)—then the interpretation may be vastly different. If the principle is accepted that each trace determines a carpel, and the reduced vascular tissue is of no importance, then the strands labelled "*x*" are carpel traces, and one half of the ventral part of each of two quite separate follicles constitutes one carpel; and the logical conclusion is reached that each follicle consists of ½ plus 1 plus ½ carpels. Perhaps it is the obvious absurdity of this necessary deduction which has thus far largely protected the Ranunculaceae and Magnoliaceae from infection by "double carpellitis."

But if the upper stelar vascular tissue is recognized for what it is, then the strands "*x*" are bundles of the receptacle, and the follicle has three traces and consists of one carpel. (It is of course necessary to the survival of the polymorphism theory that this vestigial cylinder tip of the determinate stem be "discarded" into that convenient waste basket, the pith; its recognition for what it is kills the theory.)

A further example may make this question of 'When is a trace not a trace but a receptacular bundle?' more clear.

In *Illicium* (Magnoliaceae, text fig. 21) the vascular cylinder of the receptacle does not extend beyond the ventral carpel traces. As in this plant one bundle of the receptacular stele forms all the vascular supply of a pistil, the determination of the number of traces depends upon the understanding of determinate stem tips. Either the level *a–a* or the level *b–b* represents the end of the vascular cylinder. If the former, the carpel has but one trace, which branches at once into three bundles; if the latter, then there are three traces,—one, the dorsal trace is given off at level *a–a*,



TEXT FIG. 21. Vascular system of the carpel of *Illicium anisatum* and its origin: *a*, cross section through upper center; *b*, the carpel split ventrally and spread open; *c*, the carpel seen from the side showing attachment to the receptacle. The dotted lines in the upper receptacle do not represent bundles (see text).

breaking the stelar bundle by its gap into two strands which continue up the cylinder to level *b–b* where they turn outward as the ventral traces of the carpel. (The fact that these bundles continue directly onward for some distance before turning rather abruptly outward should be evidence in itself that they are not at first traces.) If there were present vascular tissue in the position of the dotted lines, there could be no question that the point at which the ventral bundles turn outward is the point of origin of these two traces.

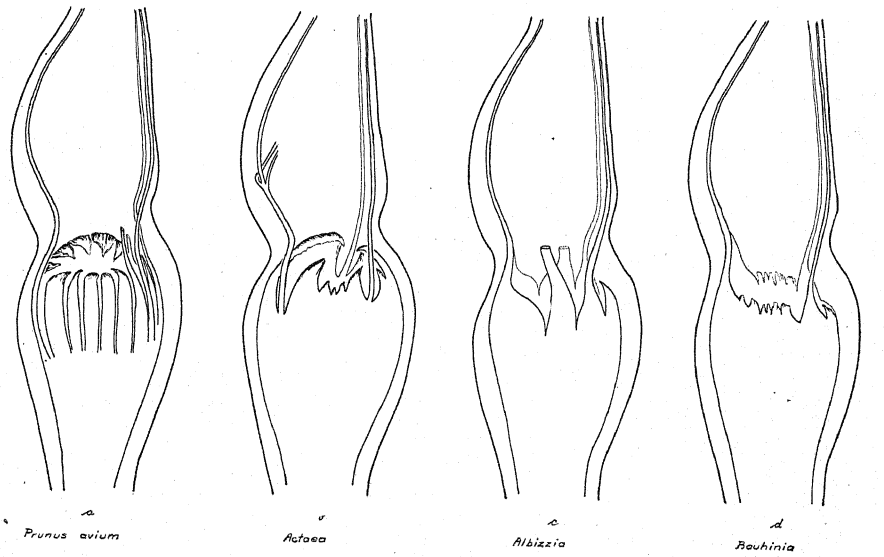
Incorrect interpretations along this line have been freely made in the interpretation under polymorphism of carpel number and type in many families, and have been a prominent contributing factor in the development of the unsound principle that every trace leading to the gynoecium determines a carpel.

The value of an understanding of the anatomy of determinate stems may appear to be of no great importance, but it becomes of critical value in the interpretation of syncarpous ovaries and of unicarpellary pistils such as those of the Leguminosae and Rosaceae.

In the case of solitary, unicarpellary pistils of the larger, non-achene types—for example, those of the Leguminosae—polymorphism states that the passage into the ovary of all the residual vascular tissue (that remaining after traces to the lower whorls have passed out) is proof that "the gynoecium not only appears to, but actually does, represent the termination of the axis." It therefore cannot consist of one carpel, as a leaf is a lateral appendage.

This is indeed a fallacious argument. If the premise were true, many foliage leaves would be terminal organs. A failure to consider normal stem anatomy is here disastrous to the argument.

Furthermore, if the pistil of *Prunus* and the follicles of the Leguminosae are terminal (as polymorphism claims), since all the residual vascular tissue goes into them, then the uppermost achene in many Rosaceous and



TEXT FIG. 22. Diagrams showing the vascular supply of the tip of the receptacle and of the carpel in unicarpellary pistils. Stages in the reduction of the "superfluous" vascular tissue are shown. (See discussion in text.)

Ranunculaceous genera is also terminal, for into it also goes all the residual vascular tissue above the lower organs.

Attention to the tips of determinate stems themselves would also have shown the inaccuracy in the premise. In such stems the behavior of the end of the vascular cylinder must be considered. In *Prunus* with five

traces (text fig. 22*a*) and *Actaea* with 3 (fig. 22*b*) the cylinder continues on beyond the exit of the traces, and the fact that the traces are not terminal on the axis is clear. In most Leguminous genera, for example *Albizzia* (fig. 22*c*), the cylinder does not extend beyond the trace origins, though in very many genera, for example *Bauhinea* (fig. 22*d*), considerable arcs of the cylinder are dropped out at that level. Even in *Albizzia*, one such tiny arc, between the ventrals, is present. It is evident that the same morphological condition is present in *Albizzia* as in *Prunus* and *Actaea;* under greater specialization the vestigial vascular tip has disappeared in these forms.

The treatment of this "superfluous" vascular tissue—the presence of which is a prominent feature of flower anatomy in so many families—as of no account, in a study which puts emphasis on vascular structure as of the greatest importance is amazing. The tissue is discarded by the proponent of polymorphism just as it is said to be by the plants, freely, with no explanation other than the teleological one that it is not needed in the formation of the carpel traces. This is not anatomy; much less is it morphology. It is teleology in the hands of, and at the command of, polymorphism.

It is certain from all kinds of taxonomic, morphological, and anatomical evidence that the solitary carpel of the Leguminosae represents the surviving member of a former whorl of carpels and that its position is a pseudoterminal one. (Completely parallel conditions occur in this family in the inflorescence, where a lateral flower in a racemose inflorescence has come to be apparently solitary and terminal.)

When two or more carpels of the original whorl are present, polymorphism is put in an awkward position. This is evident by the squirming process by which the difficulty is overcome. When the carpel number is two, resort is made to "twinning"—a process new and strange to morphology and anatomy, and somehow suggestive of genetics. Twinning is not what might be expected from its name; it is not a cleavage into two equal, like, and opposite parts, but a complex "bipartition" anatomically—exactly what occurs when the traces of two successive leaves or carpels are given off.

And what is the explanation when three carpels are present? It ought to be "triplets," of course. But no; this is said to be due to a sort of successive branching, owing to the fact that "the three radii on which the ovary primordia are laid down are not equidistant as is inevitable in a flower with a pentamerous plan." This strained and mechanistic explanation is so unnecessary; if three out of an original five spirally arranged carpels are present, the above arrangement is exactly what would occur.

Further arguments of polymorphism to prove that this pistil cannot consist of one carpel are:

That traces arising on opposite sides of a stem cannot belong to one organ; therefore there must be at least two carpels here. An acquaintance

with the anatomy of leafy stems suffices to show the error in such a statement, as has already been pointed out.

That the presence of a wedge of tissue between the pair of bundles claimed under monomorphism to be ventrals is proof that these cannot be ventrals. Again the anatomy of the leaf disproves this point, for wedges of tissue always lie between the lateral traces of leaves, small ones when these traces arise opposite the median trace. And in many flowers such wedges regularly occur between ventral carpellary traces.

3. The second of the more important errors into which polymorphism has fallen is the failure to take into account the fusion of the skeleton under cohesion. That this fusion is one of the fundamental principles in flower anatomy cannot be questioned after a broad study of flower anatomy (uninfluenced by preconceived opinions) has been made. Examples of this fusion have already been illustrated and discussed.

The case of *Saxifraga* (text fig. 19) may be returned to briefly. This ovary, as shown by its anatomy, is exactly what its external appearance suggests: two carpels fused at their base and free above. The fusion is more intimate in some species than in others, and the extent of approximation and fusion of the median laterals varies accordingly (see earlier description). The polymorphism theory claims four carpels here, two valve and two solid, interpreting the pairs of median-laterals as the midribs of the solid carpels. The solid carpels are represented, in one species, by shading. The stigmas and upper free parts are each made up of ½ plus 1 plus ½ carpels under this theory (which claims to present a more simple explanation of structure than the usual one!). All this complex interpretation is necessary because of failure to comprehend the meaning of cohesion.

The condition in *Saxifraga*, and also in the *Rutaceae* (see earlier discussion, p. 168, and text fig. 18) differs not at all from that in the Liliaceae, a family prominent in the demonstrations of polymorphism. The effect of cohesion in this family can be shown by a few examples.

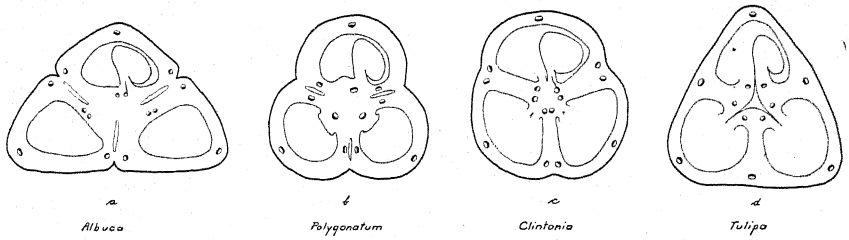
Part of the genera have carpels with three traces; others, carpels with five. In genera with five traces the median-laterals show all degrees of approximation and fusion. The septal glands—remains of the intercarpellary spaces—likewise are found in all stages of reduction. In many genera they have completely disappeared, leaving no trace.

Albuca (text fig. 23*a*) provides a good starting point. The carpel limits are evident; the septal glands are present; the median-laterals are far apart, definitely related to individual carpels, with the line of carpel union between them; the ventrals are in pairs, inverted, and unfused.

Polygonatum (text fig. 23*b*) is similar, but the median-laterals are somewhat nearer the center, and the glands separate the members of each pair. Here, according to the polymorphism theory, the solid carpel bundle is double and its halves are separated by the gland. The ventrals of each carpel are fused, so that there are but three bundles instead of six in the inner circle.

Genera with the ovary structure seen in text figure 23*c* are numerous,—for example *Ornithogalum*, *Clintonia*, *Puschkinia*, species of *Scilla*. The septal glands have disappeared, though traces remain in some genera. The pairs of bundles in the outer wall are close together, though not fused. The paired bundles are in some cases median-laterals; in others *branches* of the dorsal bundle. If polymorphism considers this last condition, it is obvious that the so-called solid and semi-solid carpels do not exist, for on its own basis, that a carpel is determined by the point of origin of its trace, these carpels then arise from another carpel.

Genera with the pairs of median-laterals fused, usually completely so



TEXT FIG. 23. Diagrammatic cross sections of ovary types in the Liliaceae. In all, 5 traces are present for each carpel. In *a*, *b*, and *c* the median laterals of adjacent carpels form pairs; in *d*, the pairs are fused. (These pairs of median laterals, fused or separate —even with the intercarpellary space between—form the midrib bundle of the solid and semi-solid carpels of polymorphism.)

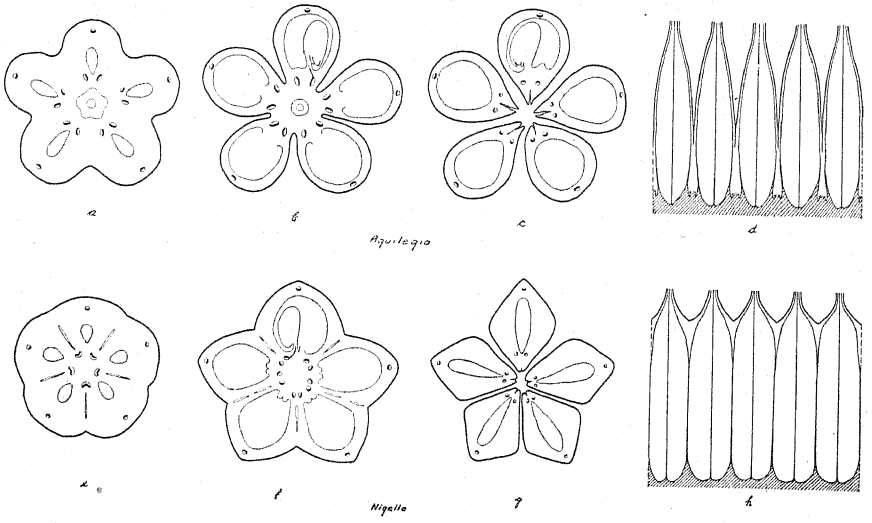
(text fig. 23*d*), constitute some of the best known forms in the family—*Lilium*, *Tulipa*, *Fritillaria*, *Erythronium*, for example.

A further example—a simple one—makes the case of the Liliaceae more clear. Polymorphism has selected from the Ranunculaceae among the follicular forms, only the syncarpous *Nigella;* just as in other families, fo-example, the Aponogetonaceae, the syncarpous form is chosen to demonr strate polymorphism, and the apocarpous follicular forms have been avoided.

If there is anywhere among Angiosperms a simple and undoubted case of syncarpy—of follicles lightly fused—it is to be found in *Nigella*. Yet this genus which is claimed to have an ovary of solid and valve carpels, is closely like many Liliaceae in anatomical structure. Let us examine the anatomy of the flower of *Nigella* and compare it with a related apocarpous genus, *Aquilegia* (text fig. 24).

Cohesion in the former is somewhat greater than in the latter, extending to a greater height. The lateral fusion is incomplete, however, and intercarpellary spaces occur. In the skeleton the slight cohesion has had no effect in *Aquilegia*, whereas in *Nigella* it has brought together the ventrals for some distance. The adjacent ventrals of separate carpels arise together (text figs. 24*e*, *h*) and continue as one for about two-thirds the length of the

loculus. Above this point the structure of the carpels is anatomically like that of *Aquilegia* at a comparable level in fusion (text figs. 24*b*, *f*). That the difference is due to the greater cohesion in one case cannot be doubted. (It is worthy of note here, as an example of the constantly occurring inconsistencies of polymorphism, that the bundles which in *Nigella* supply the ovules with traces, constitute the midrib of the solid carpel, whereas in many other families—the Liliaceae, for example—pairs of bundles similarly placed in relation to the loculi and the septa, and similarly supplying the

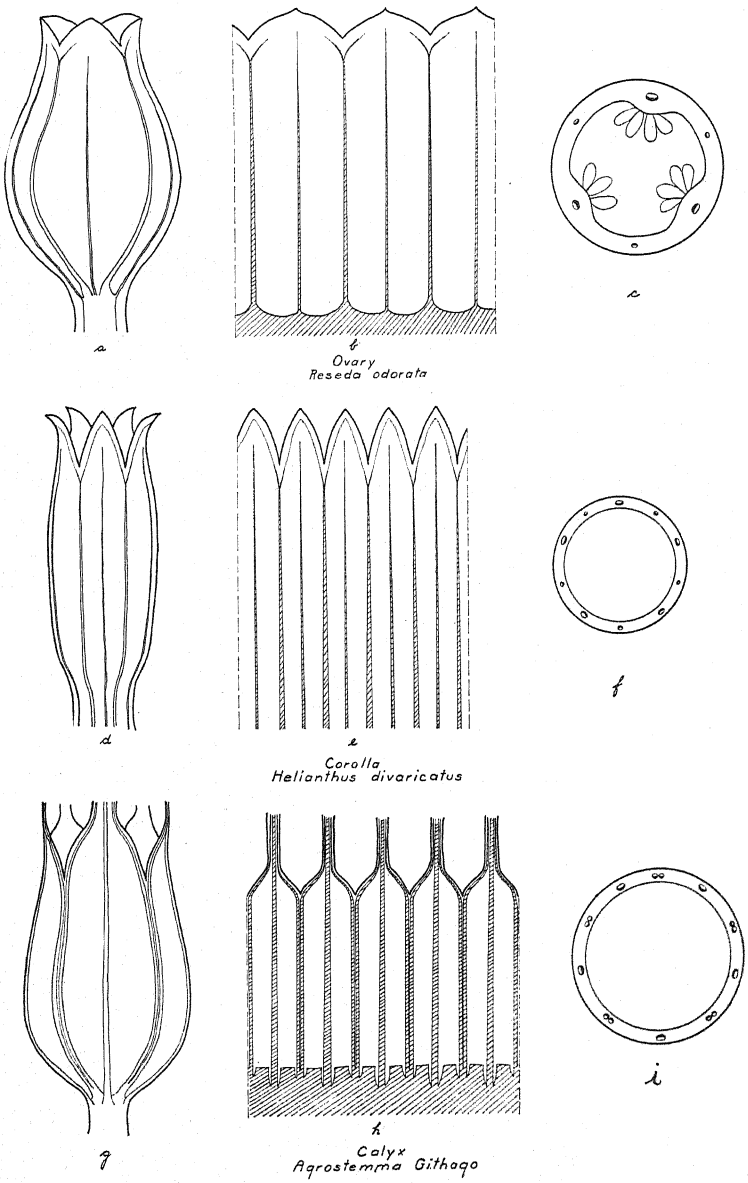


TEXT FIG. 24. Diagrams showing the closely similar anatomical structure of the gynoecium of *Aquilegia* and *Nigella*. *Aquilegia canadensis: a*, cross section showing the three traces of each carpel and the vestigial end of the receptacular stele; *b*, the syncarpous base of the gynoecium and the tip of the stele; *c*, the free follicles; *d*, the gynoecium split longitudinally and spread out, showing the origin and course of the three traces of each carpel and the origin, between the pairs of ventrals, of the strands which make up the vestigial stele. *Nigella damascena: e*, cross section showing origin of carpel traces—the pairs of ventrals of adjacent carpels arising as one; *f*, the pairs of ventrals free in the upper part of the ovary; *g*, the free tips of the carpels; *h*, the gynoecium split longitudinally and spread out, showing the origin and course of the carpel traces. The pairs of ventrals arise as one and continue fused part way up the ovary wall; above the point of separation the structure is the same as in *Aquilegia*.

ovules, are merely "placental supply," and an outer bundle or pair of bundles form the midrib.)

The genera differ further in that the vascular cylinder continues beyond the carpels in *Aquilegia* but not in *Nigella*.

This fusion in the pistil under cohesion is paralleled absolutely in the calyx and the corolla,—and not only as to lateral fusion of the veins but as to origin of the traces of these veins. Text figure 25 shows an example



TEXT FIG. 25. Diagrams to demonstrate the anatomical similarity under cohesion in ovary, corolla, and calyx, showing that the anatomical basis claimed for carpel polymorphism exists also in the corolla and calyx. Ovary, *Reseda odorata: a*, lateral view showing origin and course of traces; *b*, split longitudinally and spread out; *c*, cross section through center. Corolla, *Helianthus divaricatus: d*, lateral view; *e*, split longitudinally and spread out; *f*, cross section. Calyx, *Agrostemma Githago: g*, lateral view; *h*, split longitudinally and spread out; *i*, cross section.

from each of these whorls, demonstrating the anatomical similarity of structure. In the ovary of *Reseda* and the corolla of *Helianthus* the lateral veins are fused nearly to the sinuses; in *Agrostemma* they are fused for only a short distance, but run close together to the top. (Other genera of the Caryophyllaceae have the laterals fused to the top of the calyx, text fig. 15*b*, *c*.)

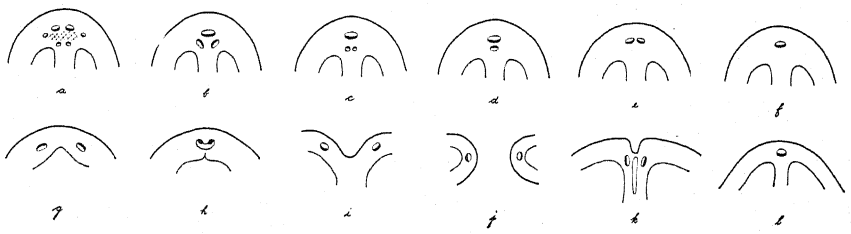
The structural condition is obviously the same. Even in the matter of fusion in origin of laterals the calyx and the ovary are alike. (The question of origin in the case of the Composite corolla cannot be considered because the ovary is inferior.)

It is now clear that if there is any logic in polymorphism, there are solid and valve sepals and solid and valve petals!

Inconsistencies

The inconsistencies of interpretation of vascular tissue under polymorphism are astonishing. They are numerous, and found in every aspect of the treatment.

Perhaps the outstanding morphological inconsistency is the acceptance of the most varied anatomy for the solid and semi-solid carpel. This



Text Fig. 26. Diagrams representing the varied anatomical structure of the solid and semi-solid carpel (see text).

organ has: a ring of bundles all facing the center of the ring (text fig. 26*a*); a pair of bundles back to back radially (fig. 26*d*); a pair of bundles side by side tangentially with phloem outward in some cases, with phloem inward in other cases (figs. 26*e*, *g*); a pair of bundles widely separated (fig. 26*i*); a pair separated by a glandular cavity (fig. 26*k*); a pair widely separated on the flanks of two nearly apocarpous pistils (fig. 26*j*); a single collateral bundle (fig. 26*l*).

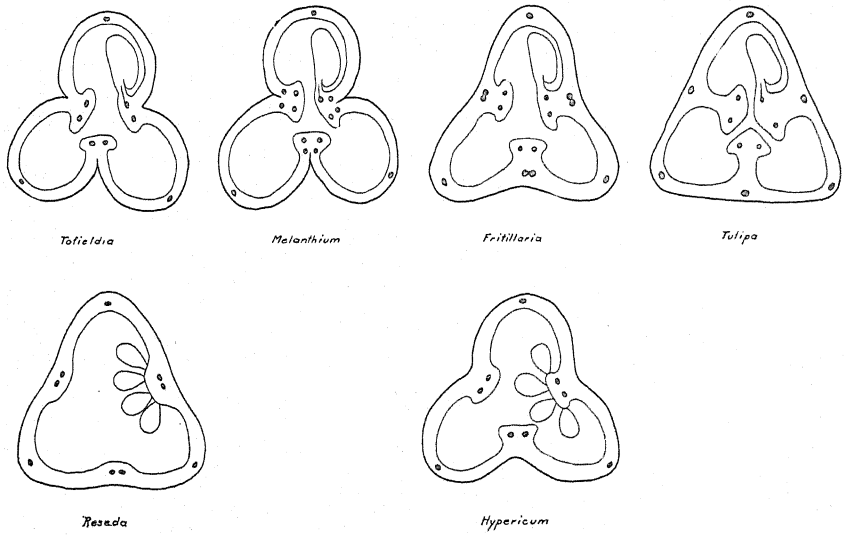
The explanations made of these fundamental anatomical differences are as amazing as the differences themselves.

In type *a* "this ring of strands becomes one bundle." "Conversion of the neighbouring ground tissue into mechanical tissue eventually gives rise to a solid fibro-vascular cord," which is apparently, under polymorphism, the morphological equivalent of the vascular bundle in the solid carpel of many families!

In the case of the Cruciferous type with two bundles side by side laterally, the anatomical structure is explained as "connected with the considerable width of the commissure, for by this arrangement the vascular bundle of the funicle of the ovule has less distance to traverse before making contact than is the case when a single median bundle is present." Again teleology is the servant of polymorphism.

The explanation of the now single, now double type of bundle in the solid and semi-solid carpel is as follows: The "double central strand . . . enables the semi-solid carpel to split at maturity down the centre between the two bundles of the midrib . . .;" that is, the double bundle is a preparation for dehiscence. Again teleology rises to the occasion. (It is worthy of note that histology informs us that the dehiscence of fruits is often without relation to vascular structure; also that in abscission, a histologically similar phenomenon, the fall of stamens, petals, leaves, flowers, fruits, and twigs does not require a previous favorable separation of vascular tissues.)

Bundles in the ovary wall which are so oriented that their xylem is



TEXT FIG. 27. Diagrammatic cross sections of ovaries showing the inconsistent treatment, under polymorphism, of the "placental supply" (see text).

toward the outside are homologous with those with phloem toward the outside, and no explanation is necessary!

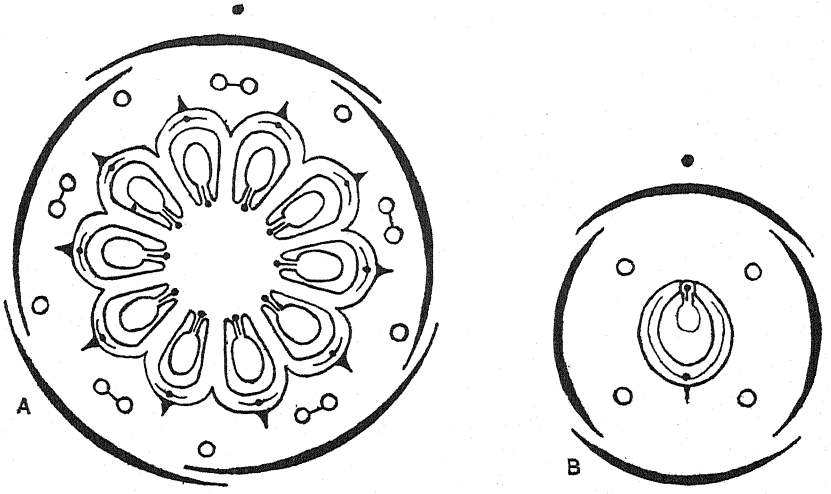
Such interpretations of anatomy are, to say the least, anomalous. This is certainly not morphology on an anatomical basis. The methods of polymorphism are, as has already been said by Parkin (1), "subversive of morphological principles."

The treatment of the "placental supply" is one of the most inconsistent

anatomical features of the theory. The pair of bundles which commonly supply the ovule traces are in some cases mere "placental supply"; in others they are the split midrib of a solid or semi-solid carpel.

A few examples will serve to make this clear (text fig. 27). In *Tofieldia* there are, according to polymorphism, three valve carpels. The vascular supply to each carpel consists of a midrib bundle and the placental supply, two bundles. The bundles of the placental supply form pairs with those of adjacent carpels.

In *Melanthium*, with a closely similar ovary, there are valve and solid carpels, the presence of the extra carpels being demonstrated by two additional bundles close behind each placental supply. These pairs of bundles represent the midrib of the solid carpel. In *Fritillaria* the midrib bundle is often double and is farther from the placental supply. In *Tulipa* the midrib is a single bundle and is far from the placental bundles.



TEXT FIG. 28. Figures after Miss Saunders, showing inconsistency in treatment of anatomy.

Even within this one family the interpretation is surprising. That the placental supply in *Melanthium* should belong to a carpel of a wholly different type and different whorl from that in *Tofieldia* is remarkable. But the inconsistency is great when these Liliaceous types are compared with similar types in other families. For example, *Reseda* (some species) is closely like *Tofieldia* anatomically; the number, position, and origin of bundles is the same. Yet the pair of bundles which supply the placenta are merely that in one family, but form the midrib of a carpel in another.

Fritillaria and *Hypericum* present a parallel case. The structure of the ovary is closely similar in these genera and the ovule traces are derived from similar pairs of bundles; yet in *Fritillaria* the bundles are placental supply only, whereas in *Hypericum* they are midrib bundles.

A simple and obvious example of the inconsistency of treatment under polymorphism is presented by the latest exposition of this theory. Compare the interpretations of genera in the Phytolaccaceae (text fig. 28). *Phytolacca decandra* is "monomorphic," each section of the syncarpous ovary consisting of a valve carpel, hence "G. 10." *Rivina humilis* is "polymorphic," the small, commonly called "apocarpous" ovary, consisting of two carpels, one valve and one solid, hence "G. 2."

Yet a glance shows the anatomy to be absolutely the same; further the origin of the traces is similar. *Rivina* has a single carpel, and hence since no single carpel can (apparently) be terminal, there must be there, as in the Rosaceae and Leguminosae, two carpels. That violence is done to anatomical consistency is of no importance here, as elsewhere, for the pronouncement has been made that ovaries of the *Rivina* type are terminal.

Complexities

The so-called "simple" theory of polymorphism has shown itself in its development to be anything but simple; its complexities are far greater than those of the accepted view. In the first place, so elaborate, various, inconsistent, obscure, and ever-changing are its bases of interpretation that apparently only the proponent of the theory can determine the structural condition in a particular case.

That the structural complexities, when explained under polymorphism, are really as great and often greater than under monomorphism may be seen from the following examples.

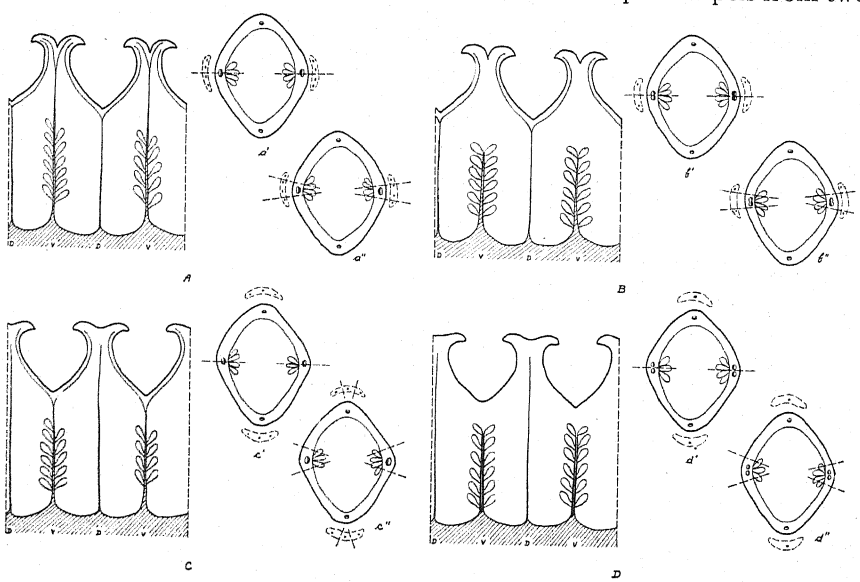
The commissural stigma stands as the "shining" example of the "difficulties" of monomorphism *vs.* the "simplicity" of polymorphism. This type of stigma has been explained by taxonomists and morphologists as made up of the united halves of stigmas normally placed which have migrated laterally and fused. This explanation—said to be based upon a "series of entirely unfounded assumptions" (are the opinions and conclusions of prominent taxonomists and morphologists to be considered as "unfounded?")—is fully substantiated by anatomical structure (text fig. 29*A*, *B*). The dorsal carpel bundles (*D*, *D*) split and the halves pass laterally to the stigmas situated midway between them (over the placentas). This is, indeed, a somewhat complex condition morphologically, but one not remarkable in any way in the range of morphological modifications in vascular plants.

Is it not strange, however, that the polymorphism theory, in the light of its objection to the above explanation, should "descend" to a completely parallel explanation of a much more common condition?

When, as commonly happens, the ventral bundles, as well as the dorsal, enter a normally placed stigma (text fig. 29*C*), polymorphism is driven to divide the tip of each semi-solid or solid carpel, pass the halves laterally to the next carpel (the same distance as in the case of the commissural stigma),

and there fuse them with the normal stigma of a valve carpel. What is impossible to monomorphism is freely accomplished by polymorphism, and the "simple" result is a stigma consisting of ½ plus 1 plus ½ carpels! The explanation of the fact that to polymorphism all things are possible is again the teleological one that the bundle splits more readily in this case because it is double in nature for purposes of dehiscence.

Not only does polymorphism present us with the "simple" condition of stigmas, styles and ovaries made up of parts of three carpels of one whorl —½ plus 1 plus ½ carpels—but with ovaries made up of carpels from two



TEXT FIG. 29. Diagrams illustrating the anatomy of the commissural stigma and the interpretations of its structure under the polymorphism theory. *A*, *B*, ovaries with commissural stigmas split longitudinally and spread open: *A*, type with ventral bundles continued beyond the placentae into the stigma; *B*, type with ventrals not extending beyond placentae. The dorsal bundle divides, one half entering the nearer half of each stigma, constituting proof of the false position and double nature of the stigma. *a'*, cross section of ovary, and position of stigma in *A*, showing the accepted interpretation of carpel number and limits—2 carpels, each stigma consisting of ½ of each carpel tip. *a''*, the same, showing the interpretation necessary under polymorphism if consideration is given to the fact that dorsal bundles enter the stigma. (Polymorphism claims, in such a case, 4 carpels, the stigmas belonging only to the 2 carpels in the placental positions.) *b'*, *b''*, similar figures of the condition in *B*.

C, *D*, ovaries with normally placed stigmas: *C*, with ventrals continuing into the stigma; *D*, with ventrals ending in the placentae. *c'*, cross section of ovary, and position of stigma in *C*, showing the usual interpretation of carpel number and limits,—2 carpels, each with a dorsally placed stigma, the tip of the carpel. *c''*, the same, showing the interpretation of polymorphism of the anatomy found in *C*,—4 carpels, each stigma consisting of ½ plus 1 plus ½ carpel tips, the tips of the solid carpels having split and migrated laterally to fuse with the tips of the valve carpels. *d'*, *d''*, similar figures of the condition in *D*.

different whorls, one above the other; and there are also ovaries made up of the members of two successive whorls of carpels, one facing anteriorly and one posteriorly. The "simplicity" of polymorphism may perhaps be merely the readiness with which any complexity "falls into line" for the proponent of polymorphism!

The polymorphism theory has accepted similarity of appearance in the skeleton of the ovary as homology. It has been led to do so by its peculiar method of development,—a working backward from high specialization to simpler forms. This method has resulted, naturally, in the interpretation of simple types in terms of advanced types, and incidentally, and of necessity, to the neglect of the fundamental principles of stem anatomy.

The unusual carpel anatomy of the crucifers, a family highly specialized in ovary structure, has been read into Angiosperms at large because of superficial resemblance. The extreme reduction condition of some of the carpels in the crucifers has suggested that similar (but morphologically quite different) structure in other families represents the same fundamental condition. Once astray along this line, the theory has "run wild" through many families, where ingenious contortions and distortions of morphological unity enable each group in turn to "fall into line and present no difficulties"—to the proponent of the theory.

The polymorphism theory is fallacious. That it has failed to establish itself is superficially evident in that it has neglected to interpret, in its terms, the follicular types about whose primitiveness no question can arise. The apocarpous follicular Magnoliaceae and Ranunculaceae are neglected. Surely these important types must have been examined. The exposition of polymorphism deals, in the Ranunculaceae, with achene types, where the pistil has the desired single trace (obviously because of reduction); it emphasizes the structure of *Nigella*, a syncarpous follicular type, but is silent about the follicular genera closely related to *Nigella*. The reason is obvious.

In the treatment of Monocotyledons, similar cases occur. In the Aponogetonaceae a syncarpous form, *Aponogeton distachyum*, is found to demonstrate polymorphism very satisfactorily (to its proponent); but conditions in closely related apocarpous species in the same genus are passed by in silence.

The theory of polymorphism is clearly unsound for many reasons. The fallacy of its development rests chiefly upon fundamental misinterpretations and upon a neglect of the anatomical principles underlying the structure of vascular plants.

Anatomy does provide evidence of the greatest value for the determination of flower morphology, but that evidence must be interpreted soundly, and in the light of established principles of comparative anatomy and comparative morphology,—not on a basis of teleology and superficial resemblance or suggestiveness.

Anatomy, however, does not claim to provide the only evidence as to the fundamental morphology of the flower; it claims only that its evidence is of the very best, because of the conservatism of vascular tissues under evolutionary modification. The determination of fundamental structure must rest ultimately upon the facts provided by taxonomy, descriptive and comparative morphology, palaeobotany, and ontogeny. A broad and sound base is built only from the varied evidence provided by all these fields of study. From none of them comes support for the theory of carpel polymorphism.

Summary

The flower is a determinate stem with appendages. The pedicel and receptacle have typical stem structure and the appendages are like leaves in their anatomy.

The sepals are, with few exceptions, anatomically like leaves. From the evidence of the vascular skeleton they are morphologically bracts and not sterile sporophylls.

The petals are anatomically sometimes like leaves, but more often are like stamens. They are morphologically sometimes leaves, like the sepals, but in most families appear to be sterile stamens.

The stamens, with rare exceptions, have a single trace. It seems probable that the single-trace condition has been derived from the three-trace condition found in a few primitive groups.

The carpels are complex anatomically. Primitively they seem to have had three traces, the one-trace and the more-than-three trace condition being derived conditions.

The effects of fusion, both cohesion and adnation, upon the vascular skeleton are demonstrated by examples. The external fusion of organs ultimately results in the fusion (phylogenetically) of those vascular tissues of the organs concerned which lie close to one another, either radially or tangentially. That is, not only are the organs or parts of organs "congenitally concrescent," but certain vascular tissues are also so fused, and evidence of this fusion may be obscure or lacking. An understanding of this is necessary to the interpretation of the morphology of specialized flowers.

When the comparative anatomy of the gynoecium is studied in very many families, that is, throughout the range of Angiosperms, it is apparent that anatomy does not support in any way the theory of carpel polymorphism.

Carpel specialization, reduction, and loss have indeed occurred freely, as anatomy gives evidence, but that modification along the lines proposed under the polymorphism theory has taken place is in no way borne out by the vascular skeleton.

The theory of polymorphism has gone far astray in its interpretations, and therefore in its conclusions, for the following reasons:

a. It fails to take into account the known anatomy of stems and leaves, neglecting the fact that a flower is a leafy stem.

b. It overlooks the determinate aspect of the flower stem and the consequent modification of the tip of the vascular cylinder, and therefore interprets bundles of the stele as traces of carpels.

c. It fails to take account of the effect of cohesion upon the vascular skeleton.

d. Its anatomical basis of interpretation is extraordinarily inconsistent.

e. It emphasizes the great value of vascular tissue in determining fundamental morphology, yet in practice freely overlooks or "discards" much such tissue.

f. It claims to explain gynoecium structure in a "simple" way and to remove all the "difficulties" of the older interpretation; yet its interpretations are as complex as—often more complex than—those of the accepted view and involve inconsistent and fantastic explanations.

g. It has mistaken analogous for homologous structure, and, taking the structural condition in a highly specialized family, has read this into simpler forms.

The theory of carpel polymorphism is clearly fallacious. It is based upon fundamental misinterpretations of stem anatomy and neglects the anatomical principles underlying the structure of vascular plants.

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MULTIPLE SEEDED SPIKELETS IN SORGHUM

R. E. KARPER

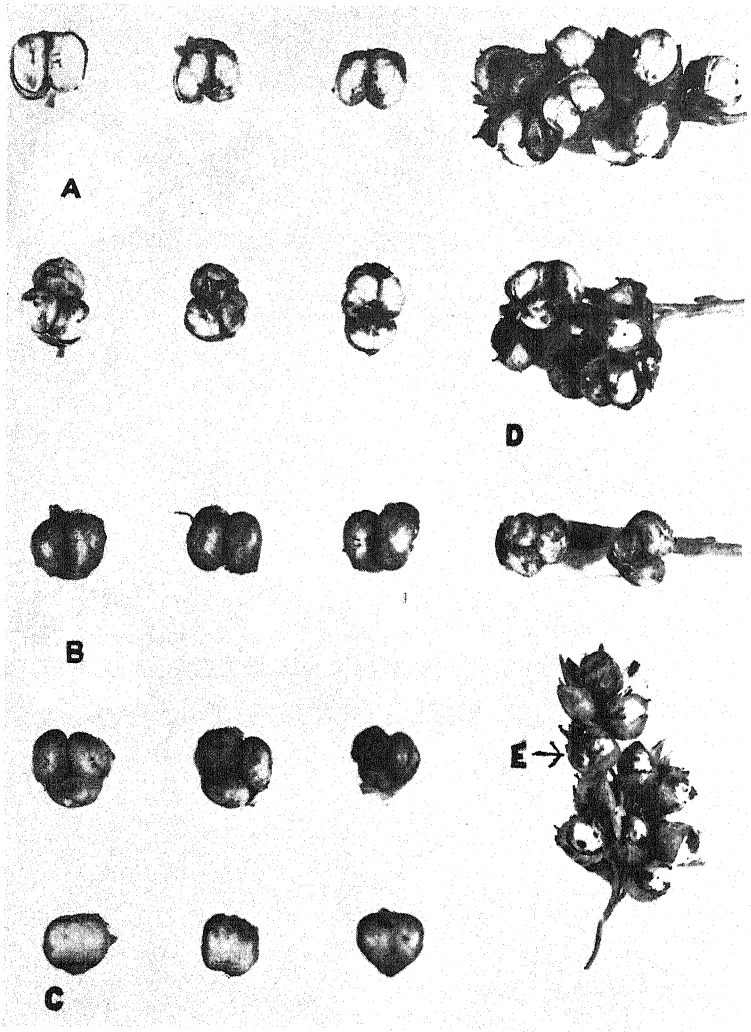
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A study of abnormalities in the multiplication or displacement of parts in plants is of value in connection with the evolutionary history of a species and may throw new light upon relationships with other species. Modifications of floral characters in sorghum are of interest from these standpoints and also furnish a clue to the morphology, location, and arrangement of rudimentary flowers in the spikelet through their occasional functioning.

The domestication of the sorghums in both India and Africa far antedates the Christian era and it is generally conceded that the cultivated forms were originally derived from the wild species of grass sorghums, which are found abundantly in tropical parts of Africa. Twin seeded spikelets of what is probably sorghum are mentioned in ancient Chinese writings and varieties of durra known to bear twin seeds are found in India at the present time. In 1915 the Office of Forage Crops Investigation found among varieties of durra imported from India a number bearing twin seeds and one spikelet with three seeds; also twin seeded and a triple seeded spikelet in sorgo. Cron (1) reports the occurrence of several triple seeded and a larger number of twin seeded spikelets in two panicles of a milo-feterita hybrid. These were thought probably to be hereditary and the seeds were planted the following year but apparently the abnormalities were not transmitted.

The writer's first observation of more than one seed in a single spikelet of sorghum was in 1921 when a single twin seeded spikelet was found in a head of milo. Later, in 1924, a profusion of twin seeds were observed in a field of milo. Notes taken at that time show that 47 percent of the heads examined in the field had some twin seeded spikelets, the number of such spikelets per head varying from one to ten. The crop was planted late, having barely time to mature before frost, and the plants had favorable moisture conditions several weeks before coming into bloom.

While taking field measurements on sorghum plants at the Lubbock station in the fall of 1928 a twin seeded spikelet of milo attracted attention. Further examination developed the fact that not only were twins present in large numbers but that spikelets with coalesced twin seeds and triplet seeds were frequently present. This condition was prevalent among both yellow and white milo, the Standard, Dwarf, and Extra Dwarf varieties, and on practically all of the panicles borne on suckers or sidebranches. Search among many other varieties of grain sorghum and also sorgos failed to reveal any multiple seeded spikelets except one kafir spikelet with twin seeds (text fig. 1).



TEXT FIG. 1. Multiple seeded spikelets of frequent occurrence in sorghum. *A*, Arrangement of twin and triple seeded spikelets in White milo; *B*, In Yellow milo; *C*, Siamese twins or connate seeds; *D*, Normal and abnormal spikelets on the seedbranches, and *E*, a twin seeded spikelet in kafir.

NORMAL INFLORESCENCE

A brief description of the normal inflorescence and of the structure of the spikelet in sorghum is essential as a basis of comparison with the abnormalities to be described later. The panicle consists of a central axis or rachis upon which are borne, in more or less definite whorls, the seed branches. Upon the branches the spikelets are borne in pairs, one sessile

and fertile and the other pedicelled and staminate, except that the terminal spikelets usually occur in threes, two of which are staminate.

The fertile spikelet has two more or less thickened and leathery glumes, the outer one partially enfolding the inner. Within these two glumes are found a perfect flower with pistil and three stamens and below it a sterile flower. The only remnant of the latter is a lemma which encloses parts of the fertile flower. The fertile flower also has a lemma of its own which bears the awn, and there is a palet, and two broad, fleshy and hairy lodicules. The pistil has two styles, smooth for the lower two-thirds of the length and then spreading into bushy stigmas. Walker (4) finds the typical *Andropogon* pistil contains three carpels, two forming the bulk of the ovary and bearing the style branches while the third is reduced to a narrow bundle which bears the ovule.

The Abnormal Spikelets

Normally the fertile spikelet of sorghum has one functional and one aborted flower. Apparently both flowers may occasionally develop and function and also, upon rare occasions, a third flower seems to be present and functional since the following fruits (text fig. 1) were observed within a single spikelet: A single kernel, the normal condition; twin seeds; connate (Kempton) seeds—a two seeded fruit formed by the fusion of parts of the two flowers; Siamese twins or semi-connate seeds—a series intermediate between twins and connate seeds; triplets—three separate kernels.

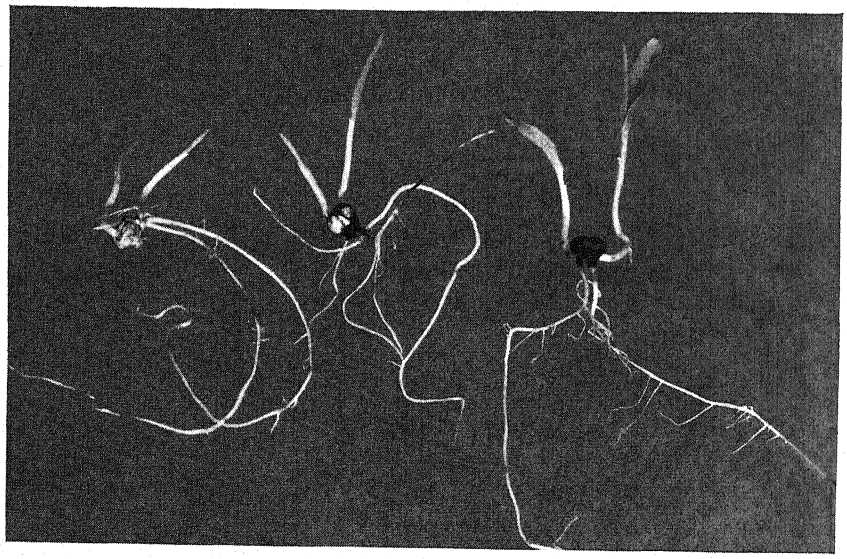
When twin seeds are borne in a single spikelet the pair of kernels are enclosed within the glumes, sometimes normally, but more often diagonally or crosswise, but are always back to back. In a single seeded or normal spikelet the caryopsis is borne with the embryo and its scutellum on the ventral or lower side next to the inner glume. When the flower which is normally abortive becomes functional the resulting kernel is inverted, having the embryo on the opposite or dorsal side. Both kernels of the pair are usually of equal size; however, one of them is sometimes slightly smaller. Unequal development is more often apparent in one of the halves of connate seeds, however, than with a member of a pair of separate kernels.

Connate seeds appear as twin kernels enclosed within the same pericarp and united back to back. These vary in size but are usually not quite as large as two separate kernels. The embryos are fully developed and when germinated connate seed produce two normal and fully developed seedlings (text fig. 2). This condition of double seeds in sorghum should not be confused with polyembryony or double embryo which has been reported in some other grass species.

Semi-connate seeds represent those in a series of variation covering the range between completely separated seeds borne as twins and completely fused seeds enclosed by a common pericarp. Coalescence is incomplete between the two seeds and may involve only a small region at the base of

the kernels while at the tip they have the appearance of two separate kernels surrounded by its own pericarp, or the union may be more extensive and approach the condition in true connate seeds where fusion is complete. Partial coalescence resulting in such Siamese twin seeds is more common than complete fusion.

Triplet seeds, while of less frequent occurrence in this material, illustrate another abnormal condition in which three functional flowers evidently developed within a single spikelet. Triplets are arranged triangularly within the glumes and in all cases noted the three kernels were separate, without any point of cohesion, and were of about equal size.



TEXT FIG. 2. Two seedlings from germinated connate seeds. It will be observed that embryos are on opposite sides.

FREQUENCY OF OCCURRENCE

Standard White milo plants, many of which had the main head blown off in a windstorm, developed shoots or sidebranches that produced heads one-half to three-fourths normal size and matured seed just before frost. In this particular variety an examination of eight heads from main plants showed 10,610 single seeded and no abnormal spikelets while twelve heads produced on sidebranches bore 5096 spikelets, 93.6 percent of which were single seeded, 5.8 percent twin seeded, .27 percent with connate seeds, and .23 percent with triple seeds. Only seven of these heads bore triple seeded spikelets and three was the largest number appearing on any one head. These abnormalities were also found on heads borne on tillers and occasionally on main heads of this and other varieties of milo.

There is a general tendency for the multiple seeded spikelets to occur more frequently toward the apex of the panicle, bearing the same relationship toward the panicle as the order of flowering, which is from the tip toward the base. Proliferation and fertilization in the early blooming apical spikelets may have caused a suppression of this tendency in the basal parts of the panicle. Classification of the spikelets in the lower half of one of the panicles in comparison with those of the apical half illustrates this distribution. Seed branches arising from the lower four nodes of the rachis embraced 80.2 percent of the total spikelets in the panicle as follows: single seeded 76 percent, twins 3.9 percent, connate seeds .3 percent, and triplets none. The upper four nodes included only 19.8 percent of the total spikelets as follows: single seeded 14 percent, twins 4.7 percent, connate seeds .8 percent, and triplets .3 percent. Since the abnormalities are not randomly distributed throughout the panicle they are probably due to a stimulus acting upon the flowers during a very brief span in their development.

Discussion

Multiple seeded spikelets of sorghum seem to occur rarely in the species grown in this country and when they do occur are much more common in milo than in other varieties. Since there is no variety of sorghum grown in this country in which multiple seeded spikelets are a common feature of development, it has not been possible to make a morphological study of such flowers and establish an accurate account of the successive developmental stages. They are abnormalities of sporadic occurrence in panicles upon which most of the spikelets are normal. Stimulation through favorable environment appears to be linked in some way with the anomalies since they were observed late in the season when temperatures were lower, daylight hours shorter, and moisture more favorable than in the normal growing season for sorghum. Furthermore, they were most numerous on heads from side shoots or plants that had the main head blown off about flowering time. Under such conditions an attempt is always made by sorghum plants to produce seed upon side shoots.

Although direct morphological evidence is lacking, several theories might be advanced in explanation of the abnormal spikelets. According to the views of Walker (4) on the structure of the pistils of some grasses, pistils of the Andropogoneae are structurally tri-carpellate, two of the carpels normally forming the bulk of the ovary and bearing the style branches while the third carpel bears the ovule. This was the typical development except in one instance, and in this case the third carpel was found to have a rudimentary style branch. If the two or three carpels failed to fuse into a single pistil but each developed an independent ovary containing an ovule, or if the carpels fused as usual into a single pistil but each carpel developed a fertile ovule, a similar series of multiple seeded spikelets could arise. In such event a single style branch remnant should

surmount the mature caryopsis, however, instead of two style branches which were actually found on each of the kernels in a spikelet and which represent the normal condition. Connate seed always had four remnant style branches, two for each member of the pair of fused kernels. This fact constitutes very good proof that the extra kernels arose from additional functional flowers within the spikelet.

The spikelets of sorghum, like those of maize and other closely related grasses, are considered as fundamentally two-flowered, the upper functional and the lower abortive. The most probable explanation of these abnormal spikelets seems to be that, under certain conditions, both flowers are functional and that, furthermore, the primordia of a third flower is at least sometimes, if not always, present in the spikelet and that this flower sometimes becomes functional.

This series of abnormal spikelets in *Andropogon Sorghum* is almost identical to a similar series in *Zea Mays* which has been described by both Kempton (2) and Stratton (3). Kempton also reports occasional development of three kernels from one spikelet in a complex hybrid of maize. Although Stratton found no triplets in *Zea Mays* var. *polysperma* where twin kernels and connate seed were common, she did find a spikelet with two functional and one aborted flowers. In sorghum, triplets were relatively common but much more rare than twins, the frequency ratio being about 25 twins to 1 triplet. The fertile flowers of the Andropogoneae are androgynous while the Maydeae are usually unisexual; however, in certain types of *Zea* the terminal inflorescence bears perfect flowered spikelets just as are found in the Andropogoneae. Some botanists feel that the common floral abnormalities in maize form almost a continuous series connecting the two tribes. Maize and sorghum apparently have many parallel genetic characters, particularly chlorophyll deficiencies, common to both genera and the vegetative characters are strikingly similar. If we consider the multiple seeded spikelets as reversions to evolutionary forms of the floral organs, the homology of these abnormalities observed in both maize and sorghum furnishes additional evidence pointing to a close relationship.

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THE MOVEMENTS OF THE LEAVES OF THE COMPASS-PLANT *LACTUCA SCARIOLA*

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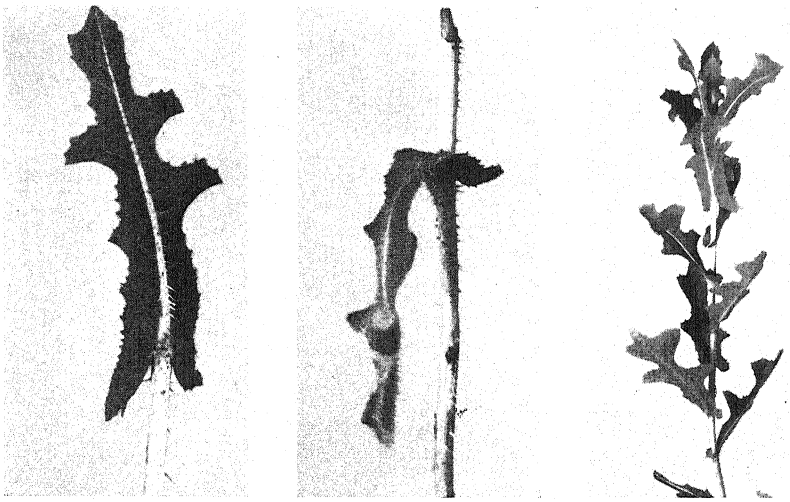
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Among the various leaf movements which have been studied, very little attention has been paid to the phototropic movements of the leaves of the so-called compass-plants. In the older American literature the phenomenon of the north-south direction of the leaves of *Silphium laciniatum* is often mentioned (Bay, 2), but little experimental work has been done with this plant. Many investigators (Erman, 3; Stocker, 12; Karsten, 9; Schanderl, 8) have considered these movements merely from an ecological point of view, the physiological basis of the movement being ignored.

According to Stahl (11), who was the first to study the leaf movements of *Lactuca scariola*, the leaves are diaphototropic, so that they tend to bring their laminae perpendicular to the sunlight. Stahl assumed that the growth conditions were favorable only during the early morning and late evening, that is, only the east and west sun would have an effect on the movements, with the result that all the laminae should point north and south. According to Mayer (5), however, the leaves twist in such a way as to place their laminae in the position in which they are equally illuminated from both sides. In nature, therefore, the leaves should only be in equilibrium if the laminae were pointing north and south. The occurrence of torsions is explained by Mayer by assuming that, if a leaf is unequally illuminated from both sides, the result will be two curvatures at different points of the leaf-base, which would combine to produce a torsion. The same explanation is given by Erman for the movements of the leaves of *Iris pseudacorus*. Seybold (9), who studied the torsions in the leaves of *Lactuca scariola*, was able to prove that the leaves of plants rotating on a clinostat were still twisted. He was, however, unable to detect the north-south orientation of the leaves, probably on account of the unfavorable weather conditions. As the conditions in Southern California are very favorable for these movements, I was able to study them further. The species used was *Lactuca scariola*, which grows abundantly in waste places. The plants were dug out and grown in pots; some experiments were also carried out with plants growing in the field.

In the case of a plant grown in the open field, all the leaves point distinctly north and south (text fig. 5). Closer inspection of the leaves shows that these positions are obtained either by torsions of the leaf base or by simple curvatures. The leaves inserted at the north and south sides of the

stem are twisted (fig. 5*A*), whereas those at the east and west sides show only curvatures (fig. 5*B* and fig. 1). The experiments were directed to determine how far these curvatures and torsions are caused by the external factors, *i.e.* the light, and how far by the internal structure of the leaves.



TEXT FIGS. 1 and 2. Explanation in text. TEXT FIG. 3. Plant illuminated from above; most of the leaves are not twisted.

Examination of the direction of the torsions in the different leaves from one plant shows that these are fairly constant (table 1).

TABLE 1. *Torsions in Two Plants Freely Exposed in the Field, June 7*

Number of Leaf from the Top	Direction of Torsion	Number of Leaf from the Top	Direction of Torsion
1	Right *	1	Right
2	Right	2	Right
3	Left	3	Left
4	Left	4	Left
5	Right	5	Right (almost none)
6	Right	6	Right
7	Left	7	Right
8	None	8	Left
9	Right	9	Right
10	Right	10	Right
11	Left	11	Left
12	None	12	Left

* For an observer looking at the point of the leaf a clock-wise torsion is here termed right, anti-clockwise left.

The same regularity was found by Seybold. This indicates that some internal factor plays a role in the movements of the leaves. Plants growing

in the shade showed the same torsions but not to the same extent and the leaves were not oriented. Some plants were rotated on the horizontal disk of a clinostat while being freely exposed to the sun. The leaves of these plants showed marked torsions (text fig. 6), thus confirming the results of Seybold. Apparently the torsions are not due to the unequal illumination but are quite autonomic.

Under which circumstances does a torsion appear? As is pointed out by Ambronn (1), Noll (6), and especially recently by Seybold (9) an organ will twist when there are three zones with different rates of growth. This applies to the leaves of *Lactuca*. The young leaves are pressed against the stem so that they stand almost vertically. They are distinctly asymmetric. One half grows faster so that the leaf curves to one side. Later on an epinastic movement starts in a direction perpendicular to the plane of the former curvature. The combination of these two movements produces a torsion in the base. At the beginning the leaves are often helical in form. Later on, however, the midrib stretches and a torsion in the base is the result. By this torsion the laminae assume the profile position. At the same time the leaves have a distinct phototropic sensitivity. Under the influence of unilateral illumination they tend to bring their laminae perpendicular to the light, as was pointed out by Stahl. These phototropic movements interfere with the torsions. When a plant is freely exposed, then apparently the sunlight in the east–west direction is more effective than in the north–south direction. (The reason for this will be discussed below.) The autonomic torsion of the leaves on the north and south sides of the stem brings the laminae perpendicular to the east–west direction. The leaves inserted at the east and west sides, however, perform a phototropic movement opposite in direction to the epinastic one, so that the latter is inhibited; that is, one of the components necessary for the occurrence of the torsion is lacking. In fact these leaves show only that curvature, which is due to the internal asymmetry (text fig. 1). In the leaves on the SW, SE, NW, and NE sides of the stem the phototropic movement is not in the same plane as the epinastic one, so that only one component of the latter will be inhibited. These leaves show therefore only a slight torsion, combined with a curvature. The inhibition of the epinasty by the phototropic movement is easily proved.

The projection on the plane of insertion [1] of the angle between the midrib of the leaf and the stem is the angle due to the epinastic movement, while the projection in the perpendicular direction depends on the asymmetric growth of the leaf. The former angle was measured. Table 2 shows that the leaves inserted at the west and east sides have the smallest angles, whereas those at the north and south sides have the largest. At the same time the angles decrease from the base to the top.

[1] The plane of insertion is the vertical plane through the stem at the point of insertion of the leaf.

TABLE 2. *Plant Growing Freely Exposed in the Field. Projection on the Plane of Insertion of the Angle Between Midrib and Stem. July 23*

Number of the Leaf from the Base	Direction of Insertion	Angle in Degrees	Number of the Leaf from the Base	Direction of Insertion	Angle in Degrees
1	NNW	80	8	SSW	55
2	E	30	9	N	50
3	SSW	75	10	SE	40
4	NE	55	11	W	25
5	SSE	60	12	ENE	35
6	W	20	13	S	55
7	ENE	30	14	NNW	50
			15	E	15

It is very easy to show that the lack of torsions in the leaves on the east and south side is due to the inhibition of the epinastic movement. If one allows a plant to wilt, the leaves will move downwards, somewhat as if under influence of the epinasty. Under these circumstances the leaves on the east and west sides also show marked torsions (text fig. 2).

Therefore it is clear that the leaves assume the north–south position under the influence of three factors: (*a*) the internal asymmetry, (*b*) the epinastic movement, (*c*) the phototropic movement.

In order to determine how far it is possible to change the direction of the leaves by changing the light conditions, the following experiments were carried out. Some plants were grown between two screens so that they could only get their light either from the east–west or from the north–south direction. The former turned all their leaves into the normal north–south position, whereas the latter moved their leaves more or less in the direction east–west, so that they were perpendicular to the direction of the light (table 3, and text fig. 7).

TABLE 3. *Plant Grown Between Two Screens so as to Receive the Light Only from the North–South Direction. Photograph Taken After 14 Days. July 3*

Number of the Leaf from Top	Direction of Insertion	Direction After 14 Days	Torsion
1	E	N–S	None
2	NW	ESE–WNW	Right
3	S	W–E	None
4	N	ESE–WNW	Left (slight)
5	SW	NE–SW	Left
6	E	W–E	Right
7	NW	E–W	Right

The orientation of the leaves in the east–west direction is not so marked as the normal north–south orientation, in plants growing freely exposed. Better results were obtained by growing a plant between two screens and illuminating it from the south side by means of a heliostat. By this arrangement the leaves received the sunlight perpendicularly during the

greater part of the day. The leaves turned markedly in the direction east–west (table 4).

TABLE 4. *Plant Grown Between Two Screens and Illuminated Daily by Means of a Heliostat from the South Side. July 29*

Number of the Leaf from Top	Direction of Insertion	Direction After 14 Days	Torsion
1	SE	E–W	Right
2	W	W–E	Left
3	NE	E–W	Right
4	S	E–W	None
5	NW	WNW–ESE	Right
6	E	SE–NW	Right
7	SW	W–E	Left
8	NE	E–W	Left
9	S	E–W	None

In this case the leaves inserted on the east and west sides are twisted, whereas those on the north and south sides are only curved to one side by the asymmetrical growth, the epinastic movement being inhibited.

Some plants were grown in such a way that they were shaded from all sides, getting their light only from above. Under these circumstances there was no orientation of the leaves at all. Most of them did not even twist (table 5 and text fig. 3).

TABLE 5. *Two Plants Illuminated from Above. Photographed After 14 Days. July 14*

Number of the Leaf from Top	Direction of Insertion	Torsion	Number of the Leaf from Top	Direction of Insertion	Torsion
1	W	None	1	SW	None
2	SE	None	2	E	Slight left
3	N	None	3	NW	Slight left
4	SW	None	4	SSE	None
5	ENE	None	5	NNE	None
6	WNW	Slight right	6	W	Slight right
7	SSE	None	7	SE	Slight left
8	NNE	None	8	NNE	Slight left
9	WSW	Slight left	9	WSW	None
10	E	None	10	SE	None

The absence of torsions requires an explanation, since in this case the epinasty is not inhibited by a phototropic movement. On examining the leaves it appeared that they had quite a different structure. The laminae were thinner and the mechanical tissues were less developed than in the normal ones. They had more of the character of shade leaves. In these leaves the asymmetrical structure had also more or less disappeared and this is the reason why, notwithstanding the epinasty, they twisted only slightly.

In order to determine the reason why the light in the east–west direction is so much more effective than in the north–south direction, some growth

measurements were carried out. If the hypothesis of Stahl is true and the leaves do not grow during the middle of the day, then it would be easy to understand that the light in the south direction is ineffective. The length of some leaves was measured in the morning as well as in the evening and from these data the average increase was calculated (table 6).

TABLE 6. *Growth Measurements of Four Leaves. The Figures Give the Length and the Increase in Millimeters*

Leaf	17 VI 10 A.M.	17 VI 6 P.M.	Incr. per hour	18 VI 9 A.M.	Incr. per hour	18 VI 5:30 P.M.	Incr. per hour	19 VI 9 A.M.	Incr. per hour	19 VI 5:30 P.M.	Incr. per hour	20 VI 9 A.M.	Incr. per hour
B_1	45	45.5	0.06	49	0.23	50	0.12	53	0.19	53.5	0.06	56.5	0.19
B_2	42	43	0.13	47	0.27	48	0.12	52	0.26	53	0.12	58	0.32
C	33	34	0.13	36	0.13	37	0.12	40.5	0.23	41	0.06	44	0.19
A	36	36.5	0.06	39	0.17	39.5	0.06	40	0.03	40.5	0.06	42.5	0.13

During the night the leaves grow about twice as fast, but there is still a distinct growth during the middle of the day. It is therefore rather improbable that the unfavorable growth conditions during the middle of the day could be the cause of the north–south direction of the leaves. It is, however, easy to understand why the leaves assume the north–south direction, by considering the total amounts of sunlight that fall on the vertical planes in the direction north–south and east–west. Almost all investigators who have worked with compass-plants assume that, at least at higher latitudes, the leaves get the smallest amount of sunlight by pointing north and south. According to them the ecological significance of this should be a diminution of the transpiration and a protection from too strong insolation. Stocker pointed out that for lower latitudes this cannot be true, but according to him the plants should show in these regions a tendency to bring their leaves in the east–west direction, so that they should still be protected from the intensive sunlight. This statement has, however, been criticized by Seybold (10), who was unable to find the east–west direction at low latitudes.

If we consider a vertical plane standing in the north–south direction, then the amount of sunlight falling at any moment on unit surface will be:

$$E_1' = E\varphi \cos \alpha \cos \beta$$

where E is the amount of sunlight falling on a horizontal plane under the conditions when the atmospheric absorption is negligible (sun in zenith), φ is the factor for the atmospheric absorption and varies with α, α is the altitude, and β the azimuth.

The total amount of sunlight will be:

$$E_1 = \sum E_1'$$

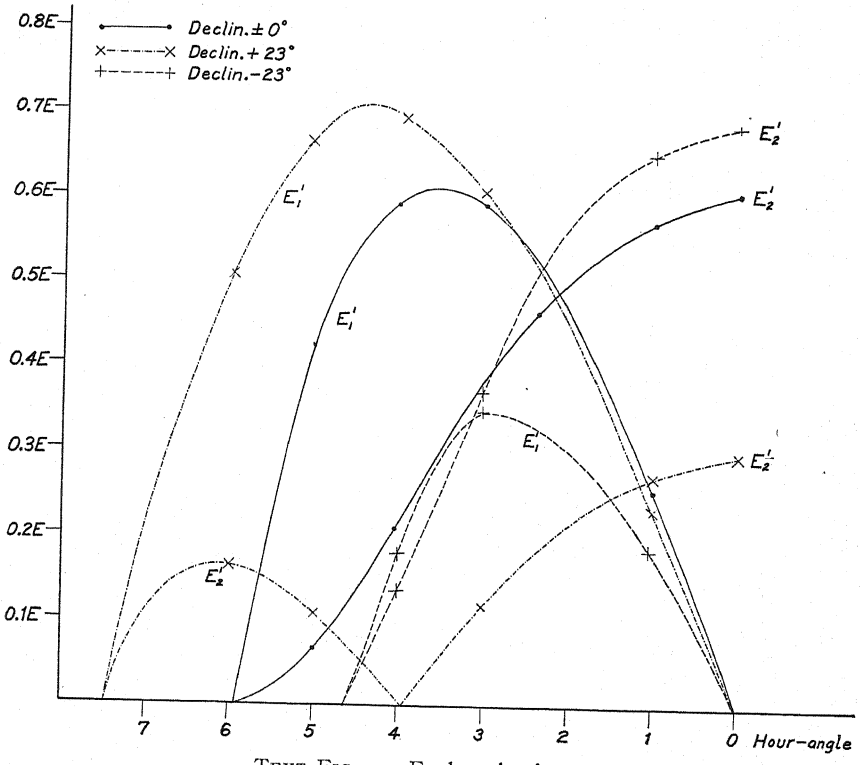
The amount falling at any moment on a vertical plane standing in the east–west direction is:

$$E_2' = E\varphi \cos \alpha \sin \beta$$

and the total amount:

$$E_2 = \sum E_2'$$

The easiest way to calculate E_1 and E_2 is to calculate for different hour-angles of the sun the values of E_1' and E_2' and to plot these in a curve. By measuring the areas of these curves with a planimeter one can obtain



TEXT FIG. 4. Explanation in text.

the values of E_1 and E_2 fairly exactly. In text figure 4 the curves for different declinations of the sun at a latitude of 40° are given (but only between sunrise and noon).[2]

The type of the curves obtained in this way varied with the latitude. Values of E_1 and E_2, calculated from the curves for different latitudes and different declinations of the sun, are given in the following table.

[2] The values of φ were taken from Müller, "Die Photometrie der Gestirne," Leipzig, 1897; the values of α and β from the tables of "The Sumner line of celestial bodies," Hydrographic Office, Washington, 1924.

TABLE 7. *Total Amounts of Sunlight Falling on Unit Surface of Vertical Planes in the Directions North–South (E_1) and East–West (E_2). The Intensity E During One Hour is Taken as Unity*

Latitude	Declination of the Sun									
	+23		+10		0		−10		−23	
	E_1	E_2	E_1	E_2	E_1	E_2	E_1	E_2	E_1	E_2
0	22.5	18.0	25.0	8.0	25.5	0	25.0	8.0	22.5	18.0
10	25.5	13.3	25.8	2.0	25.8	6.0	23.0	13.5	19.0	20.5
30	29.8	5.3	27.3	10.3	23.8	16.5	18.8	21.3	13.5	23.0
40	33.0	10.8	27.8	15.5	22.0	20.3	16.0	21.5	9.3	19.8
50	34.5	15.5	27.5	19.8	19.5	23.0	13.5	21.5	4.8	14.0
60	38.0	21.8	26.6	23.0	17.3	22.5	8.3	17.3	1.8	4.5

It is clear from this table that at all latitudes up to 60° the plane pointing north and south receives much more sunlight in the late spring and mid-summer than the one pointing east and west. Furthermore the intensity of the light striking the east–west plane at noon is not larger than the maximum intensity of the light striking the north–south plane (see for example text fig. 4). In winter time it is, however, just the reverse. Especially at higher latitudes the east–west plane now receives much more light than the north–south one, but this has no direct significance, since no compass-plant grows during this time. As it has already been shown that the leaves tend to bring their laminae perpendicular to the direct sunlight, the north-south direction is thus explained.

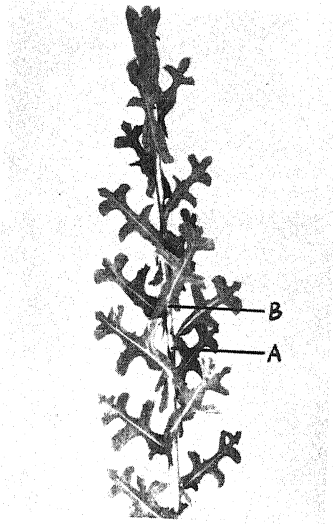
If this explanation is right, then it should be possible to alter the direction of the leaves by intensifying the light in the south direction. This was tried by illuminating a plant from the south side during the middle of the day with a heliostat; the east and west sides received the normal amount of sunlight. As is shown in table 8 most of the leaves point east and west (see also text fig. 8).

TABLE 8. *Plant Illuminated Daily from 9:00 A.M.–3:00 P.M. with a Heliostat from the South Side. Photographed After 14 Days. July 7*

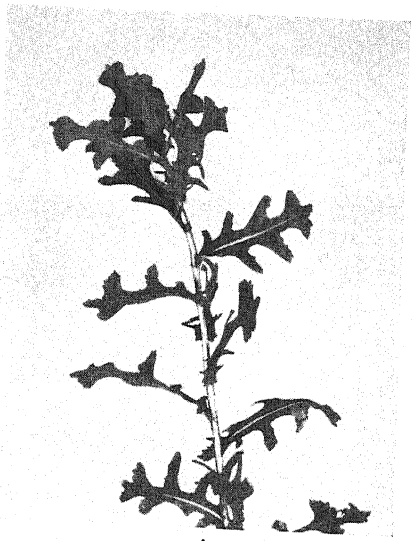
Number of the Leaf from Top	Direction of Insertion	Direction after 14 Days	Torsion
1	S	W–E	None
2	NW	WSW–ENE	Left
3	E	NW–SE	Slight left
4	SW	E–W	Left
5	N	W–E	None
6	SE	W–E	Right
7	W	N–S	None
8	NE	W–E	Left

The same phenomenon ought to take place when a plant is grown in winter-time, provided the sunlight conditions are favorable enough to produce any phototropic effect.[3]

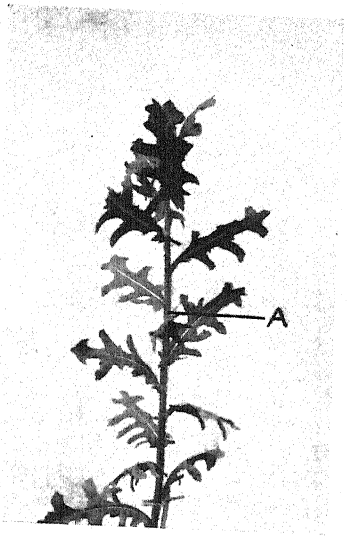
[3] Some experiments carried out in the greenhouse this winter showed that this was in fact the case.



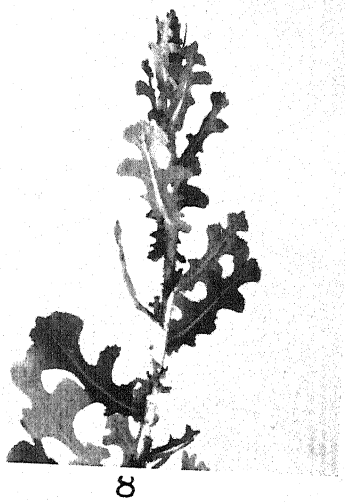
5



6



7



8

TEXT FIG. 5. Plant grown in the field. The leaves inserted at the north and south sides are twisted (*A*), whereas those on the east and west sides show only curvatures (*B*). Photographed from the east side. FIG. 6. Torsions in the leaves of a plant rotated on a clinostat. FIG. 7. Plant grown between two screens so that it was illuminated only from the north and south sides. Only the leaves above *A* were developed under these conditions. Photographed from the south side. FIG. 8. Plant illuminated from the south side by means of a heliostat; most of the leaves are pointing east–west. Photographed from the south side.

Whether the north–south direction of the leaves has any ecological significance is hard to say. A decrease in transpiration or a protection from too strong insolation is out of the question as the leaves do not point in the direction of the strongest light. It is better not to speculate about this as it is quite possible that the north–south direction has no ecological significance at all.

Summary

1. The movements of the leaves of *Lactuca scariola* have been studied under various conditions.

2. It is shown that the leaves are twisted in the base and that the torsion is due to an autonomic asymmetric growth and an epinastic movement.

3. The leaves tend to bring their laminae perpendicular to the direct sunlight.

4. Under certain circumstances the epinastic movement is inhibited by the phototropic one, so that no torsion takes place.

5. As the total amount of sunlight in the east–west direction is larger than in the north–south direction, the leaves assume the north–south direction, thus receiving the maximum amount of sunlight.

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NOTES ON THE TECHNIQUE OF MEASURING THE GROWTH OF BACTERIA WITH A NEPHELOMETER

MARY WOTHERSPOON COLLEY

(Received for publication October 7, 1930)

The purpose of the following paper is to present to students of bacteriology certain points relative to the use of the nephelometer in measuring the turbidity of bacterial cultures.

In a series of experiments, the writer used the Kober nephelometer, made by the Klett Manufacturing Company. Yoe (1929) has most adequately described the general development of this instrument. An account of the particular nephelometer used by the writer in her experiments is given by Kober (1917, 1918), Kober and Klett (1921), and Yoe (1929).

While the nephelometer has been developed primarily by chemists, its application to bacteriological and mycological problems has been advocated by Colley (1926), Dienert (1914), Hockenyos (1928), Hoefer (1922), McFarland (1907), Riker (1926), and Roubal (1927).

Somewhat different problems confront the chemist and bacteriologist in the use of the nephelometer. According to Yoe (1929) one should first see if the chemical suspension or solution to be tested obeys Beer's law, *i.e.*, the reading for one-half the standard should be exactly twice that of the standard, and the reading of one-third the standard should be exactly three times that of the standard. If the solution does not obey this law, a nephelometric curve must be constructed which, when applied to the readings, will render the amount of light reflected strictly proportional to the weight of the precipitate under observation. It is claimed by some investigators that certain instruments are so perfect in construction that the use of this correction constant is unnecessary. Chemists using the Kober nephelometer are advised to adjust their readings to attain the highest possible accuracy.

On the other hand, the bacteriologist, working with ever fluctuating living things, might find the use of correction constants a superfluous and confusing refinement. The following experiment was made in order to see to what extent Beer's law held for suspensions of bacteria tested in the Kober instrument.

A heavily seeded twenty-four-hour culture of *Pseudomonas tumefaciens* Smith and Town, grown in a colorless liquid synthetic medium (see Riker, 1926), was thoroughly emulsified by shaking. The amounts indicated below were removed with a graduated 5 cc. pipette, and added to distilled water in a series of ten test tubes, as follows:

Tube	Bacterial Emulsion (cc.)	Distilled Water (cc.)
1	10	0
2	9	1
3	8	2
4	7	3
5	6	4
6	5	5
7	4	6
8	3	7
9	2	8
10	1	9

Each of these ten tubes was used in turn as the standard (set in the nephelometer at 15) and in rotation, all the other tubes were compared with it. The nephelometric readings so obtained are given under *A* in table 1, and the corresponding calculated values under *B*.

When the figures under *A* and *B* are compared one with the other, it will be seen that a greater accuracy is obtained in the more concentrated emulsion, and where there is not too great a difference in the number of organisms in the contrasting tubes. However, despite the fact that the dilutions were made very roughly, and the figures represent just one set of readings, *A* and *B* parallel each other fairly closely, and the figures indicate that, with care, the method has definite possibilities for measuring growth differences in bacterial cultures. Beer's law is seen to hold with sufficient consistency to indicate that with certain refinements in technique, and with experience, it is better to consider the readings proportional to the turbidity in the solutions and obtain the averages of many readings from many cultures, rather than to refine the method to a point where the value of the correction constants would fall below the limits of experimental error.

Certain points in technique, developed in the writer's experience with the nephelometer in bacteriological work, are given below.

The establishment of the standard to be used, against which to compare the cultures of unknown bacterial content, presents the same difficulty as with all turbidity measurements. Control cultures may be used if one is running a series of experiments, but unless the control itself is standardized, the results obtained will be purely relative, *i.e.*, percent increase or decrease compared with the control. While such information is frequently all that is desired, it is possible to standardize the control approximately by plating out and counting the number of organisms, or by comparing it with tubes of standard turbidity the bacterial count of which has been previously determined (Hotchkiss, 1923). Instead of using bacterial cultures as standards, turbid chemical suspensions have been found satisfactory (Hockenyos, 1928; McFarland, 1907). The use of ground glass has been suggested by Kleinmann (1925). The writer used control cultures as standards. Since it was not easy to grasp the relation of an unknown culture to the control

TABLE I. *Comparison of Nephelometric Readings (A) with Calculated Values (B) for Pseudomonas tumefaciens Suspensions*

Number of cc. of Bacterial Emulsion Compared with Standard	Number of cc. of Bacterial Emulsion Used for Standard																			
	10		9		8		7		6		5		4		3		2		1	
	A	*B*	*A*	*B*	*A*	*B*	*A*	*B*	*A*	*B*	*A*	*B*	*A*	*B*	*A*	*B*	*A*	*B*	*A*	*B*
10	15.0	15.0	13.9	13.5	12.7	12.0	11.5	10.5	10.0	9.0	9.8	7.5	8.0	6.0	6.5	4.5	5.2	3.0	3.5	1.5
9	17.0	16.7	15.0	15.0	13.8	13.3	12.4	11.7	10.7	10.0	10.0	8.3	8.2	6.6	7.1	5.0	5.2	3.3	3.5	1.7
8	18.8	18.8	17.3	16.9	15.0	15.0	13.3	13.1	11.9	11.3	11.0	9.4	9.1	7.5	7.7	5.6	5.7	3.8	4.2	1.9
7	21.0	21.4	19.5	19.3	18.1	17.1	15.0	15.0	13.6	12.8	12.0	10.7	10.4	8.6	8.5	6.4	6.2	4.3	3.9	2.1
6	24.0	25.0	22.0	22.5	20.1	20.0	18.0	17.5	15.0	15.0	14.0	12.5	11.8	10.0	9.6	7.5	7.7	5.0	4.0	2.5
5	29.5	30.0	26.4	27.0	24.0	24.0	20.2	21.0	18.2	18.0	15.0	15.0	13.2	12.0	10.8	9.0	8.0	6.0	4.6	3.0
4	37.0	37.5	32.8	33.3	29.2	30.0	24.7	26.2	22.3	22.5	20.0	18.8	15.0	15.0	12.8	11.3	9.4	7.5	5.5	3.8
3		50.0	44.5	45.0	37.0	40.0	32.0	35.0	30.4	30.0	26.1	25.0	20.1	20.0	15.0	15.0	11.7	10.0	6.7	5.0
2		75.0		67.5		60.0	47.5	52.5	43.0	45.0	34.7	37.5	27.7	30.0	23.5	22.5	15.0	15.0	8.8	7.5
1		150.0		135.0		120.0		105.0		90.0		75.0	48.1	60.0	41.4	45.0	28.5	30.0	15.0	15.0

from the nephelometric readings, these readings were first recorded, and then converted into percents of the control, which was established in each case as 100 percent, by means of specially prepared constant charts.

As Denis (1921) and Kober (1918) have already pointed out, the work is best done in a darkened room. It is well to check up frequently on the standard solution, as in the case of the "O" on an analytical balance. The bacteria may be grown in a clear colorless liquid medium (preferably synthetic) or on solid media and subsequently emulsified with water or sterile saline (Roubal, 1927). The former, for practical reasons, is to be preferred, and is that used by the writer in her researches.

All turbidity which affects the light passing through the nephelometer, and therefore the readings themselves, should be due directly to the organisms. Anything that affects the passage of the light, such as the presence of bacteria of unequal size, uneven distribution of the organisms, the presence of a foreign colloid or precipitate, or color in the medium, interferes with the accuracy of the readings.

Obviously, the bacteria should be as nearly as practicable the same size. Where large forms, having definite stages in their life cycle (*e.g. Azotobacter*) are being studied, the work may be simplified by using young cultures, in which the organisms are still in the motile stage, or by confining the measurements to older cultures, all of which are approximately the same age.

Of equal importance with the size factor is the factor of even distribution, This condition of homogeneity usually obtains in young broth cultures. Where a sediment or pellicle has formed, however, it is necessary either to heat (Broadhurst, 1924) or to shake thoroughly to insure an even distribution.

If there is a foreign precipitate present, it is sometimes possible to dissolve it by the addition of substances such as dilute acid. But if this procedure is followed, care must be taken that the material added does not cause an agglutination of the organisms or in any way alter their nephelometric value.

If the liquid medium has a faint uniform color in all tubes, the accuracy of the readings is not likely to be greatly affected, especially after the technique of handling the cultures has been acquired. If color differences do exist, however, their influence may be greatly minimized by the use of suitable color screens, or by proper dilutions of the cultures before examination.

Cultures of non-pathogenic organisms can be tested directly without further treatment. However, where the nephelometer is used with virulent organisms, the tubes may be subjected to heat or certain chemicals in small quantities, to bring about sterilization. One should guard against the addition of substances which will alter the nephelometric readings (Roubal, 1927).

The whole culture may be placed in the cup for examination, or if it is desired to keep it growing over a long period of time, small portions may be

removed at intervals, made up to the necessary volume with water or saline, and tested nephelometrically. Care must be taken, of course, to use equal amounts of the cultures and to dilute with equal amounts of water for any one series of examinations.

As a check on the nephelometric method it will usually be found advisable to make occasional plate cultures and counts from both the control cultures and the cultures to be compared with the controls. The ratios of the living organisms in the cultures to the living organisms in the control tubes may be compared with similar ratios deduced from the nephelometric readings. The latter figures, however, are often to be regarded as indices of the total number of the dead and living bacteria present.

TABLE 2. *Growth of Pseudomonas tumefaciens in a Colorless Liquid Synthetic Medium Containing Dilute Solutions of $MnSO_4$*

Number of Days Incubated	2		5		10	
	A	*B*	*A*	*B*	*A*	*B*
Control	100	100	100	100	100	100
.00005 N	101	101	99	91	98	100
.0001 N	108	99	105	84	104	98
.0005 N	118	112	128	95	109	76
.001 N	107	111	106	84	102	63
.005 N	62	51	99	74	96	69
.01 N	55	60	59	37	61	30

A = Relative turbidity measured nephelometrically.
B = Relative number of colonies obtained by plating and counting.
Each figure represents the average of eight cultures.

The results of such a comparison are shown in table 2 in which the controls for plate counts and turbidity are set at 100 percent. As would be expected, the two sets run nearly parallel during the period of most active cell division. Deviations appear in older cultures where there is an accumulation of dead protoplasm, and in some cases at least of decomposition products. Indeed, the turbidity ratios give a picture of growth more as one thinks of it in the higher plants, where the size of the specimen is due not only to the cells that are viable at any one particular time, but also to the dead cells that are present along with the living.

The writer has found that the nephelometer offers a speedy and accurate means of comparing the turbidity of bacterial cultures, with the personal equation reduced to a minimum. Detailed results of a series of experiments will appear in a later paper.

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CULTURE EXPERIMENTS WITH *PSEUDOMONAS TUMEFACIENS*

MARY WOTHERSPOON COLLEY

(Received for publication October 7, 1930)

The following notes are based on the results of some preliminary experiments undertaken to find the optimum conditions for the growth of *Pseudomonas tumefaciens* Smith and Town in a liquid synthetic medium.

The original culture was obtained in 1923 from Dr. M. Levine of the Montefiore Hospital, New York, N. Y. The medium employed was made up as follows:

Monobasic potassium phosphate (Merck's Sorensen's)	0.5 gms.
Magnesium sulfate	0.2 "
Dextrose (bacto)	1.0 "
Asparagine	1.0 "
Water to	1000 cc.

The pH was adjusted, except where otherwise indicated, to 6.5 by the addition of sodium hydroxid, after which the medium was sterilized in the autoclave at fifteen pounds pressure for ten minutes. Ordinary tube cultures, containing 10 cc. of this medium, became cloudy in twenty-four hours.

The experiments described below were conducted to determine how slight changes in the medium and in the cultural conditions affected the growth of the bacillus. Growth was measured with a Kober nephelometer (Colley, 1931) and by check plate counts. In general, the experiments showed that with the hydrogen-ion concentration initially somewhere between pH 6.0 and 8.0, growth increased as the temperature rose to 28° C., when the oxygen supply was abundant, and when the quantity of dextrose was increased.

The Initial Hydrogen-ion Concentration. Tubes adjusted to varying hydrogen-ion concentrations (pH 5.0–8.0) before inoculation, after two days incubation still showed a varying pH. However, the pH became more or less uniform, approximately 7.6, after ten days. When the amount of dextrose was increased to 1.0 percent, the pH after ten days averaged 6.6. The relative amount of free acid as determined by titration was also found to vary with the sugar content, increasing proportionally with the amount of dextrose in the medium.

Incubation Temperature. That the optimum temperature for *Ps. tumefaciens* grown in the synthetic media used was nearer 28° C. than 22° C. was shown both by nephelometric readings and plate counts. (Cf. Riker 1926, and Smith, 1911.) The nephelometer revealed a growth increase for

28° C. compared with 22° C. as a standard, of 98 percent for five days, 92 percent for seven days and 81 percent for ten days. Plate counts showed an even greater difference than that indicated by the nephelometer.

Both the 22° C. and the 28° C. cultures at the end of the ten day period contained many viable organisms. In the 22° C. cultures the media was by no means exhausted, and it was relatively free from toxic by-products. In the 28° C. cultures the sugar was occasionally exhausted but the cultures were by no means "by."

Oxygen Tension. The sensitiveness of *Ps. tumefaciens* to various oxygen tensions was determined as follows: In preliminary experiments tubes of unequal diameter were used, and, of course, the available oxygen supply varied with the cross sectional area of the tube cavity. The growth in the wider tubes averaged approximately 33 percent above that which took place in the narrower ones. When 10 cc. quantities of media were placed in 50 cc. Erlenmeyer flasks, inoculated, and incubated at 28° C., nephelometric tests showed an increase of 172 percent in two days over identical material in media tubes, and an increase of 29 percent after five days. Plate counts showed an increase of 285 percent after two days, and 50 percent after five days. These results are interpreted to mean that cell division proceeds far more rapidly where an abundance of oxygen is available, resulting, however, in an exhaustion of the media and in the probable production of metabolic by-products toxic to the organisms themselves.

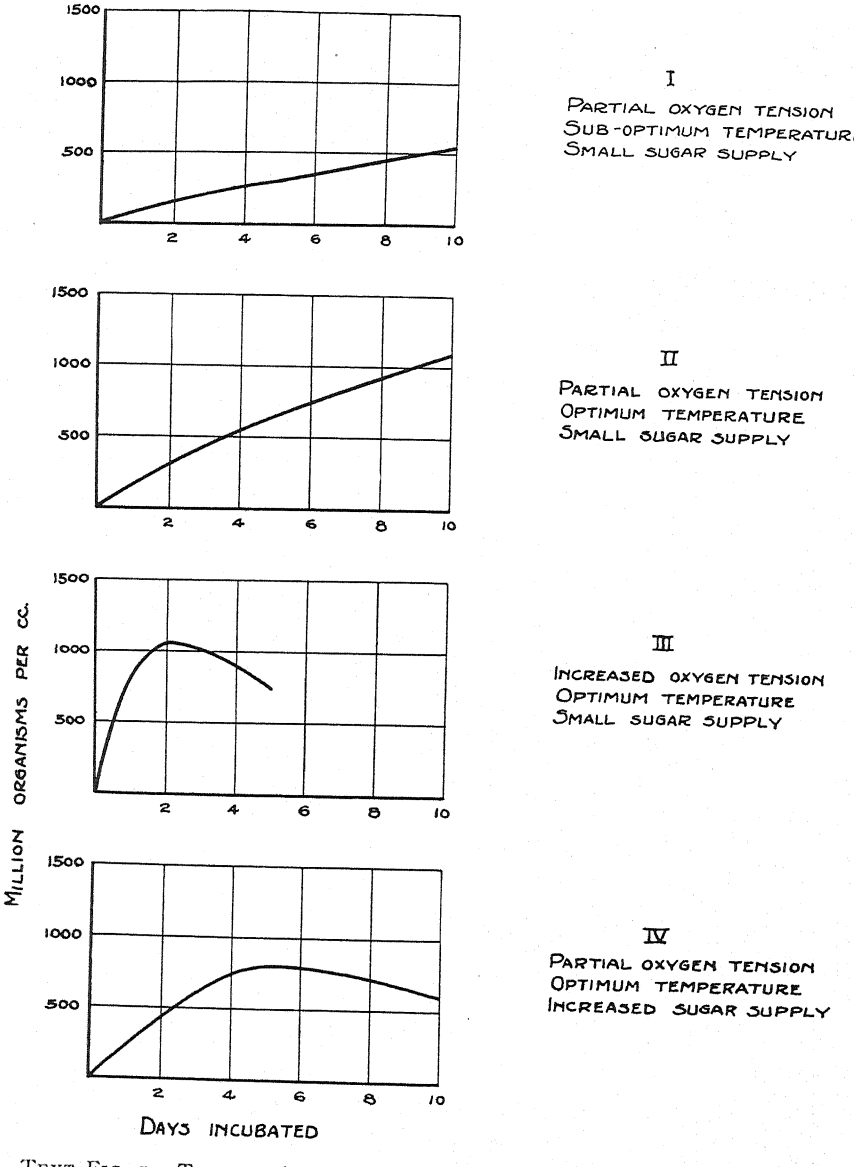
Dextrose. Tube cultures containing originally 0.1 percent dextrose were shown by the Fehling test to contain sugar after ten days incubation at 22° C. After ten days incubation at 28° C. sugar was sometimes but not always present. Where 10 cc. of media had been placed in 50 cc. Erlenmeyer flasks and incubated at 28° C., the dextrose had completely disappeared after five days. Cultures with 0.5 and 1.0 percent dextrose were grown in test tubes at 28° C., and up to ten days the Fehling test for sugar was always positive.

A series of tubes containing 10 cc. of media varying only in the dextrose content (0.1, 0.5 and 1.0 percents), incubated at 28° C., showed after two days an increased turbidity of 30 percent in the 0.5 percent dextrose and a 60 percent increase in the 1.0 percent dextrose over the 0.1 percent; after five days a 24 percent increase in the 0.5 percent dextrose and a 60 percent increase in the 1.0 percent; after ten days a 14 percent increase in the 0.5 percent dextrose and a 7 percent increase in the 1.0 percent.

It was found by averaging the results of many series, that a tube culture with 0.1 percent dextrose, having after inoculation approximately 17,500,000 organisms per cc., incubated at 28° C., contained after two days 360,000,000, after five days 685,000,000, after seven days 885,000,000, and after ten days 1,075,000,000 viable organisms per cc.

The amount of growth which occurred with specific variants is indicated in type graphs based on plate counts shown in text figure 1. The areas

under the curves may be taken as measures of the number of organisms produced during the life of the culture. The investigator will be likely to inquire which of these curves is to be considered the optimum growth curve. The question naturally arises whether optimum conditions are those under which the maximum number of organisms are produced at a given time in



TEXT FIG. 1. Type graphs to show effect of varying environment on growth of *Pseudomonas tumefaciens* in a synthetic medium.

the life of the culture, or those producing the maximum number during the life cycle of the culture. Curve III, for example, illustrates the optimum conditions for the production of the greatest number of organisms in the medium two days after making the inoculation, while Curve II indicates the optimum conditions for producing the greatest number of organisms during the culture cycle.

For the most part the writer adopted for detailed experiments culture conditions from which growth curves comparable to Curve II could be obtained. The results of these experiments will appear in a later paper.

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STUDIES ON THE SENSITIVITY OF *MIMOSA PUDICA* II. THE EFFECT OF ANIMAL ANESTHETICS AND CERTAIN OTHER COMPOUNDS UPON SEISMONIC SENSITIVITY[1]

RAYMOND H. WALLACE

(Received for publication July 26, 1930)

The preceding paper of this series described in some detail the prevention of the sleep movements of *Mimosa pudica* and other plants by means of ether and acetylene, as well as the unsuccessful attempts to do likewise with nitrous oxid, ethylene, and chloroform. The present paper will present the results of a series of experiments on the effect of animal anesthetics and other compounds upon the sensitivity of *Mimosa*.

The sensitivity of *Mimosa pudica* to physical and chemical stimulation is the basis for the popular term "sensitive plant," and constitutes one of the most striking evidences, but by no means the most delicate one, that the plant is a living organism. It is perhaps worth while to quote in full the very early description of this species by Mayo (1827):

> If a terminal subleaflet be pinched with forceps, or cut with scissors, it rises together with its fellow; then the next pair rise; then the next; and so on in succession, till all the pairs of subleaflets upon the same subpetiole are folded. In a little time afterwards, the petiole is bent downwards at its intumescence; and in a few seconds more the remaining leaflets upon the same petiole fold their subleaflets in pairs, from the base toward the point of the leaflet. If a subleaflet be burnt, instead of being cut or pinched, the phenomena above described occur more rapidly; and after they have taken place, the adjoining leaves upon the same branch are bent downwards in succession, their leaflets brought together, and their subleaflets folded. If the plant is very vigorous and lively, an impression made upon one leaf affects the rest in succession. It is well known that the stem, branches, flowers, and roots of the sensitive plant have no motion. But M. Desfontaines observed that, on touching the roots with sulphuric acid, the leaves become folded; and M. Dutrochet obtained a similar result on burning either the flowers or the stem.

MATERIALS AND METHODS

The experiments reported below upon the effects of various chemical compounds on the sensitivity of *Mimosa pudica* are both qualitative and quantitative. The qualitative ones consisted essentially in the application of the methods used in the studies on sleep movements to an analogous series on sensitivity.

It is essential, before quantitative data can be obtained, to determine both the physiologically significant range of concentrations for each com-

[1] This paper is the second of a series of three appearing in successive issues of the Journal.

pound, and the length of exposure suitable. The time consumed in qualitative experiments was in most cases many times greater than that finally used in obtaining the quantitative data upon which the graphs for the compounds are based. This is especially true for the tests on ether and chloroform.

The plants used were young and vigorous with for the most part four mature leaves, like those used in the studies on sleep movements. In order to secure optimum conditions of light and temperature, the tests were made in a very large greenhouse between May first and October first. The temperature for each experiment was never less than 25° C. and frequently it was 35° and 40° C. Most of the experiments were run during the middle of the day, from 10 A.M. to 4 P.M.

This series of experiments was made with twelve of the more significant chemical compounds. The first five of these—ether, chloroform, nitrous oxid, acetylene, and ethylene—were used in the experiments on sleep movements. Carbon tetrachlorid and carbon disulphid were tested because of their chemical similarity to chloroform. Five alcohols—ethyl, methyl, propyl, butyl, and amyl—were likewise tested. Table 4 shows the volume

TABLE 4. *The Number of Cubic Centimeters of Various Liquids Required to Give a Vapor Concentration of 1 Percent in a 20 Liter Container*

Compound			Cc. of Liquid Required	Physiologically Significant Range of Concentration	Presentation Times
Name	Mol. Wt.	Density			
Ether	74	.72	.80	2 to 25 percent	5 min. to 24 hrs.
Chloroform	120	1.5	.62	.5 " 3 "	2 to 30 min.
Carbon tetrachlorid	154	1.6	.76	.5 " 4 "	" " " "
Carbon disulphid	76	1.26	.46	1.0 " 10 "	" " " "
Methyl alcohol	32	.80	.32	1.0 " 10 "	" " " "
Ethyl alcohol	46	.79	.49	.2 " 5 "	Less than 15 min.
Propyl alchol	60	.80	.60	Less than 1 percent	" " " "
Butyl alcohol	74	.88	.66	Less than 1 percent	" " " "
Amyl alcohol	88	.82	.82	Less than 1 percent	" " " "

The physiological range of concentration given in this table is the approximate range which was found to be significant in these studies; it extends from concentrations having little or no effect on the plants through to those which seriously injured or killed the plants within a few minutes.

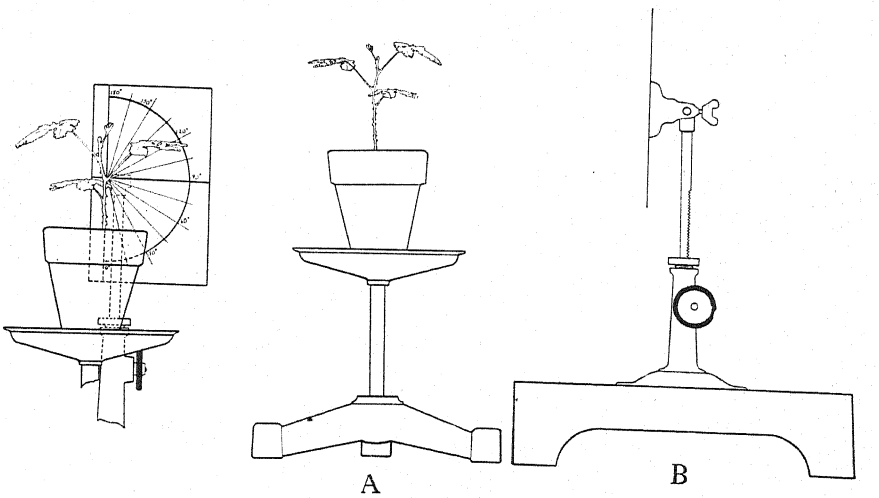
of the liquids which will give a vapor concentration of 1 percent when allowed to evaporate in a twenty-liter belljar at a temperature of 30° C. This table also shows the physiologically significant range of concentration for each compound, together with the suitable presentation times.

Only chemically pure compounds were used in making up the various concentrations of vapor. The necessity for this precaution was well demonstrated in experiments in which anesthetic ether (about 5 percent alcohol

present) was tested, with results which were quite different from those found with pure ether. This is discussed in some detail below.

The sensitivity of *Mimosa pudica* to physical and chemical stimulation may be measured in at least four ways. It may be expressed as the intensity of stimulus which will induce a reaction in the primary, secondary, or tertiary pulvini; or the rate of transmission of this stimulus along the petioles or stems; or the time required for a pulvinus to complete its response; or, finally, it may be the angle through which primary, secondary, or tertiary petioles move upon stimulation. In all of the subsequent studies, unless stated to the contrary, I shall use the angle of movement of the primary petiole in recording my results. The angle of movement of the secondary or tertiary petioles might be used as a measure of sensitivity, but the physical difficulties involved in the determinations preclude this.

The angle of movement of the primary petiole recorded in the tables



TEXT FIG. 1. Face view of the protractor scale showing petiole number 2 superimposed on the scale, and giving the initial position of the petiole.

TEXT FIG. 2. Diagram of entire apparatus used for determining the sensitivity of the primary petioles of *Mimosa pudica* (for full explanation see text).

and graphs was measured by means of the apparatus shown in text figures 1 and 2. This apparatus consists of two principal parts, a turn-table, "*A*," and a ratchet and pinion, "*B*," upon which is mounted a protractor. One places the plant on the turn-table and sights across the base of the petiole toward the protractor scale, and carefully raises or lowers the protractor, by means of the ratchet and pinion, until the 90 degree line of the scale just cuts the top of the pulvinus being measured, as shown in text

figure 1. The initial position of petiole number one, the outside angle which the petiole makes with the stem upon which it is borne, is then read off in degrees. The turn-table is then moved until petiole number two is parallel to the plane of the protractor scale, and the position of this petiole is read. The positions of petioles number three and four are then determined in the same manner. After the initial positions of all four petioles are determined, the plant is stimulated vigorously by jarring and the final positions of each petiole determined in the same manner. The difference between the initial and final positions is the angle of movement.

The protractor used in this apparatus was drawn very carefully on a piece of heavy cardboard. It was calibrated to an angle of 2.5 degrees, the divisions being drawn in with inks of various colors to facilitate the reading of the scale. The angles can be read to within 2.5 degrees with little difficulty, but this would not be possible if the various colors were not used. The accuracy of the apparatus was tested by the close agreement in readings taken by different persons.

A point of even greater importance in these studies than the absolute accuracy of the individual readings is the speed with which the determinations can be made. If a plant has been exposed to a given concentration of a certain compound for a definite length of time, it is very necessary to know that the readings have been made before the effect of the substance has had time to change appreciably. This possible error was held at the minimum in these experiments because all readings were usually complete within 45 to 60 seconds after the plant was removed from the exposure jar.

The complete procedure for the preliminary experiments described below may be stated briefly as follows. A vigorous *Mimosa* plant with four or more mature leaves was chosen for each concentration in a particular series of the compound being studied. The plant was placed in the belljar and the required volume of liquid or gas liberated into the chamber in the manner already described. The belljar then remained sealed for a given length of time. At the end of this time the chamber was opened and the general appearance of the plant and the state of its sensitivity were noted. In addition, subsequent examinations extending over a period of several days were made to determine whether or not injury had occurred. Other series were made testing various combinations of concentration and exposure time.

The procedure for the final quantitative experiments was similar to that for the preliminary ones. In this case, however, only the physiologically significant concentrations and suitable exposure times within the limits of injury were used. A set of five plants each having four mature leaves was used to test each concentration of each compound. It will be understood, therefore, that each value in each table and hence each point on each curve represents an average given by twenty petioles on five individual plants. The entire series of experiments on concentration was run simultaneously, one

plant to a concentration. The series was then repeated four times so that the set of five plants for each concentration was used one after the other. The plants were left in the exposure chambers for the desired length of time; they were then carefully removed from the jars, placed on the turn-table, and their angles of movement determined.

Results

Ether

It was obvious from the first preliminary experiments that the seismonic sensitivity of the primary, secondary, and tertiary pulvini of *Mimosa pudica* could be completely destroyed by a certain range of concentrations of ether vapor.

A series of more than twenty experiments made between May 1st and September 1st showed that concentrations of ether vapor less than 10 percent were usually insufficient to prevent the movements of *Mimosa*. Thus when plants were exposed to 5, 6, 7, 8, and 9 percent vapor, the primary, secondary, and tertiary pulvini continued to respond when stimulated. The intensity of the response is greatly reduced, however. Very commonly the secondary leaflets remained entirely expanded after stimulation in the next higher concentration, 10 percent. In concentrations of 12 and 13 percent the pulvini of these leaflets were always insensitive, and even the primary pulvini were practically so. It would seem therefore that the pulvini of the secondary leaflets usually become insensitive at a slightly lower concentration of vapor than that necessary to destroy the sensitivity of the primary pulvini.

The time required for complete loss of sensitivity may vary greatly. Usually the secondary leaflets were rendered insensitive in three to ten minutes, depending upon the concentration and other factors. A period of twenty to thirty minutes is usually sufficient to anesthetize the primary pulvini of fresh vigorous plants, but for others an hour, an hour and a half, or even two hours may be required. I have even found conditions during the fall and winter when primary petioles could not be anesthetized at all, using any combination of concentration and exposure time. I have never found a condition, however, when the secondary leaflets could not be anesthetized in a few minutes.

The fact that ether would usually insensitize *Mimosa* plants with ease under some conditions and would entirely fail to do so under others was the most peculiar phenomenon encountered in these studies. From May 1st, when the first tests were made, until September 1st, when the last of the quantitative data given below were obtained, they reacted in a uniform manner. Two weeks later, however, September 15th, I found it impossible to induce complete insensitivity by any concentration of ether, even though the plants were exposed to the vapor for more than an hour.

These two weeks intervening between the time when the plants could be

anesthetized and when they could not be anesthetized were a period of very dark rainy weather when the light intensity was often insufficient to cause normal expansion of the leaflets during the day. Likewise it occurred at about the time that the greenhouse became shaded by a tall building across the street, which reduced the direct sunlight falling on the greenhouse to only a few hours during the middle of the day. I was convinced, therefore, that even though the plants appeared entirely normal they must have been affected by the reduced light, or some other condition of which I was not aware.

I then grew new plants by means of supplementary artificial illumination, described in more detail in the next paper of this series. The plants grown in this way could be anesthetized completely by the usual concentrations of ether, but the exposure time was never quite as short as in the plants grown in the greenhouse between May 1st and September 1st. A thirty minute exposure was, however, sufficient in most cases. Unless specifically stated to the contrary, all plants used in the test described below are those grown from May 1st to September 1st.

Tests with long exposure times from one to eight hours have indicated that those concentrations too weak to cause insensitivity within the usual exposure times will not generally cause insensitivity if the length of exposure is increased. Concentrations less than 11 percent never anesthetized the primary petioles; those of 11 and 12 percent might cause insensitivity; and 13 percent always anesthetized.

There is usually a slight stiffness or sluggishness in the reaction of plants which have been exposed for several hours to concentrations between 8 and 16 percent, but it gradually disappears after a few days and no evidence of permanent injury remains. It is possible that this sluggishness due to long exposure has been interpreted by some investigators as anesthesia, but it appears to be a more or less transitory paralysis. This will be described more fully in connection with chloroform. Plants which have been exposed to concentrations of 22 percent or more for several hours may show permanent partial paralysis or other evidences of injury.

In my paper on sleep movements there are photographs showing that a completely anesthetized *Mimosa* plant is practically identical in appearance with a normal plant for the same time of day. If the plant is exposed to ether during the day when the leaflets are entirely expanded and the petioles up, the plant remains in exactly this position after anesthetization. If, on the other hand, the plant is exposed to the ether in the morning before the plant has assumed its day position, the leaflets and petioles remain in the night position. As can be seen in the photographs, the only visible effect of the anesthetic upon the plant is a peculiar twisting and curving of the immature leaves and shoots. This condition is only temporary, disappearing soon after the plants are removed to the open air.

Attempts to anesthetize a *Mimosa* plant in the position which it assumes

after stimulation have been without success. The leaves always return to their original position before they are insensitized. This is perhaps to be expected, however, since maximum time for recovery from stimulation rarely ever exceeds the time required for ether to insensitize the plants. I have tested this point by placing a plant in strong ether vapor (30 percent) for five minutes, the plant being still sensitive. I then stimulated the plant and it took the reacted position, after which I replaced it in a concentration of 15 percent vapor. Thirty minutes later the plant was insensitive, but most of the primary petioles had only partly recovered their former positions and in many tests almost all the secondary leaflets were practically closed. It seems very probable, therefore, that a plant could be insensitized in the stimulated position if the anesthetic could be made to act in less time than is required for the plant to recover from stimulation. The fact that the petioles may continue to rise even though the plant will not respond to stimulation may be of no significance, since it is well known that the petioles are insensitive for a short time subsequent to reacting even when no anesthetic is present.

TABLE 5. *The Effect of Various Concentrations of Ether upon a Set of Plants Tested on Three Successive Days*

Date	Temp. (F.)	Percent of Vapor	Position of Petioles		Angle of Movement
			Initial	Final	
Aug. 28....	90	0.0	135.0	69.2	65.8
		2.5	143.8	85.5	58.3
		5.0	141.0	104.5	36.5
		7.5	143.6	129.8	13.7
		10.0	141.5	135.6	6.0
		13.0	138.6	138.6	.0
Aug. 29....	84	0.0	133.7	71.1	62.0
		2.5	142.3	92.5	49.8
		5.0	135.5	100.7	34.8
		7.5	133.6	119.6	14.0
		10.0	134.8	127.8	7.0
		13.0	131.0	131.0	.0
Aug. 30....	80	0.0	118.1	64.0	54.1
		2.5	135.8	86.5	49.3
		5.0	127.8	98.8	29.0
		7.5	132.8	117.8	15.0
		10.0	120.9	113.7	7.2
		13.0	117.9	117.9	.0

The concentrations are given as percent of vapor to gas volume of the containers.

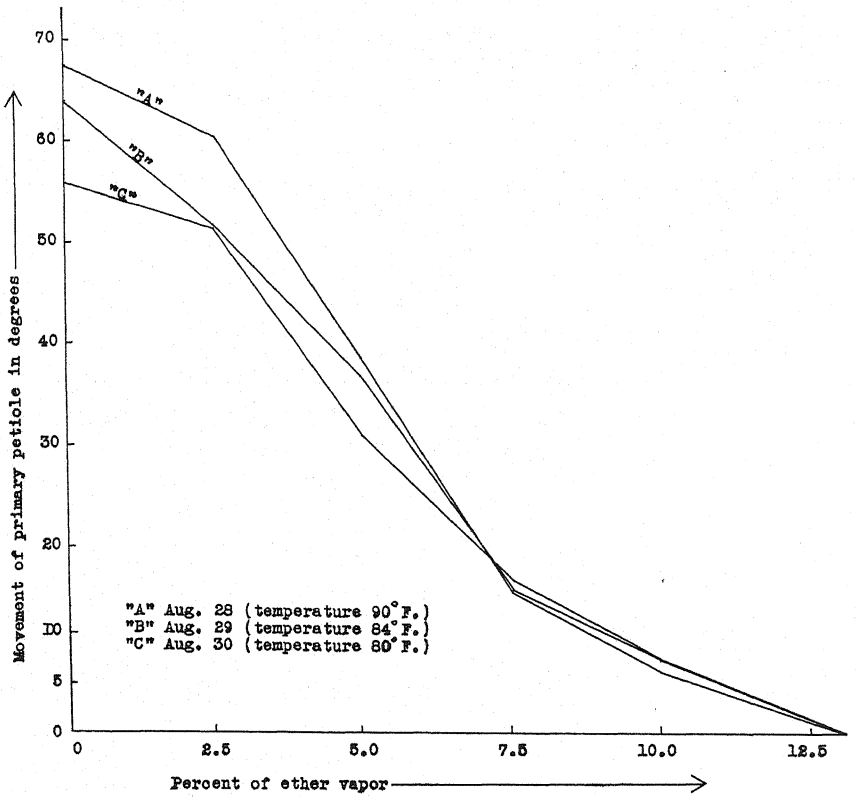
Each value given in this table for initial position, final position, and angle of movement is an average of the readings on twenty petioles on five different plants.

Exposure time, 30 minutes.

In text figure 3 and table 5 are presented the quantitative data as to the angle of movement of the primary petioles after 30 minute exposures to

from 2.5 to 13 percent ether vapor. This combination of concentration and exposure time was known to be suitable from the preliminary experiments. The exposure time was actually ten minutes longer than that necessary at that time to anesthetize the plants by 13 percent ether. A set of five plants was chosen for each concentration, and an additional set of five for control. The experiment was run first on August 28th and then repeated on the same plants on August 29th and 30th to determine whether there were any apparent after effects from the ether treatment.

As can be seen in the graph there is a spread in the curves at 0, 2.5 and



TEXT FIG. 3. The effect of various concentrations of ether vapor upon the sensitivity of a set of *Mimosa* plants tested on three successive days. Data from table 5.

5 percent ether, but they approximate each other very closely in the concentrations of 7.5, 10 and 13 percent. The initial spread would seem to indicate a decreasing gradient of sensitivity following each successive exposure to the vapor. This is, however, only an expression of variation in sensitivity in accordance with temperature differences for the three exposure periods. The temperatures for the three tests were as follows:

August 28th, 90° F.; August 29th, 84° F.; August 30th, 80° F. As not a leaf discolored either during or within three days after the last test, there was no qualitative evidence of injury. If, however, anesthetic ether (4 percent alcohol) was used, the plants often exhibited definite injury in the form of slight discoloration around the margin of the leaflets. Further experiments showed this to be due entirely to the alcohol present.

The loss of sensitivity in various concentrations of ether follows the usual S curve. The lowest concentration tested in the quantitative experiments reduced the sensitivity by about 12 percent. An increase in concentration to 5 percent resulted in a marked reduction of sensitivity to 50 percent normal. It might be said in passing that a loss of 50 percent in sensitivity is visible by ordinary observation. The curve flattens out gradually in 7.5, 10, and 12.5 percent and finally approximates zero at a concentration of 13 percent. I have tried many times to anesthetize with 12 percent ether, but have been able to do so only a few times and in those cases only with excessively long exposure times. It would seem therefore that 13 percent is the minimal concentration which can be considered as anesthetizing *Mimosa pudica*. Still deeper anesthesia can be induced by concentrations up to about 25 percent, but in concentrations above that, permanent injury or death usually results.

The plants will recover partial sensitivity very quickly after removal from the exposure chambers. As already stated in my paper on sleep movements, the secondary leaflets react slightly to stimulation within a minute after removal and are apparently almost back to normal in five minutes. The secondary and primary pulvini respond slightly in about fifteen minutes, but several hours are required to bring them back to normal.

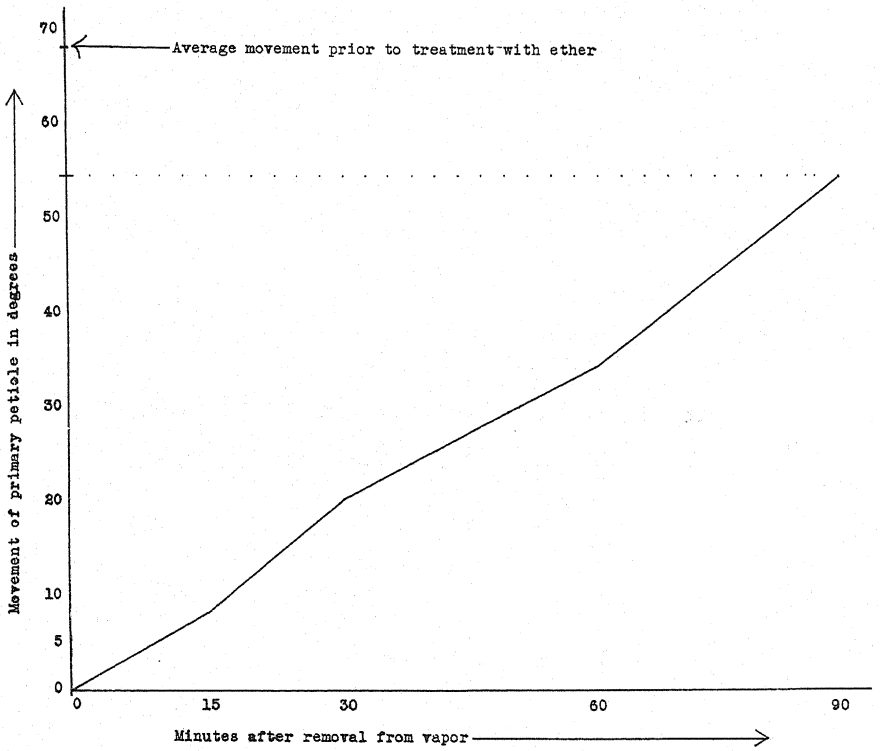
TABLE 6. *The Return of Sensitivity to the Primary Petioles of Mimosa pudica after Having Been Treated with 15 Percent Ether for 30 Minutes*

Plant No.	Angle of Movement before Anesthetizing	Angle of Movement after Removal from Vapor				
		0 min.	15 min.	30 min.	60 min.	90 min.
1	70	0	11	15	22	44
2	70	0	7	36	45	59
3	66	1	9	10	29	45
4	66	0	9	15	33	53
5	68	0	5	25	43	68
Average	68	.2	8	20	34	54

Experiment begun at 2 P.M. at a temperature of 85° F.

This is shown in text figure 4 (and table 6) where the angle of movement for returning sensitivity is plotted against time elapsing after removal from vapor. Five plants gave an average angle of movement of 68 degrees just prior to being placed in belljars in the presence of 15 percent ether vapor.

On removal 30 minutes later the plants were entirely insensitive. Fifteen minutes after removal, however, they were beginning to recover and moved an angle of 8 degrees on stimulation. After thirty, sixty, and ninety minutes, the sensitivity showed an increasing gradient of 20, 34, and 54 degrees, respectively. The angle of movement was, however, still 14 degrees below normal after one and one-half hours. It is interesting to note that when returning sensitivity is plotted against time elapsing after removal from the ether vapor, the resulting curve very closely approximates a straight line.

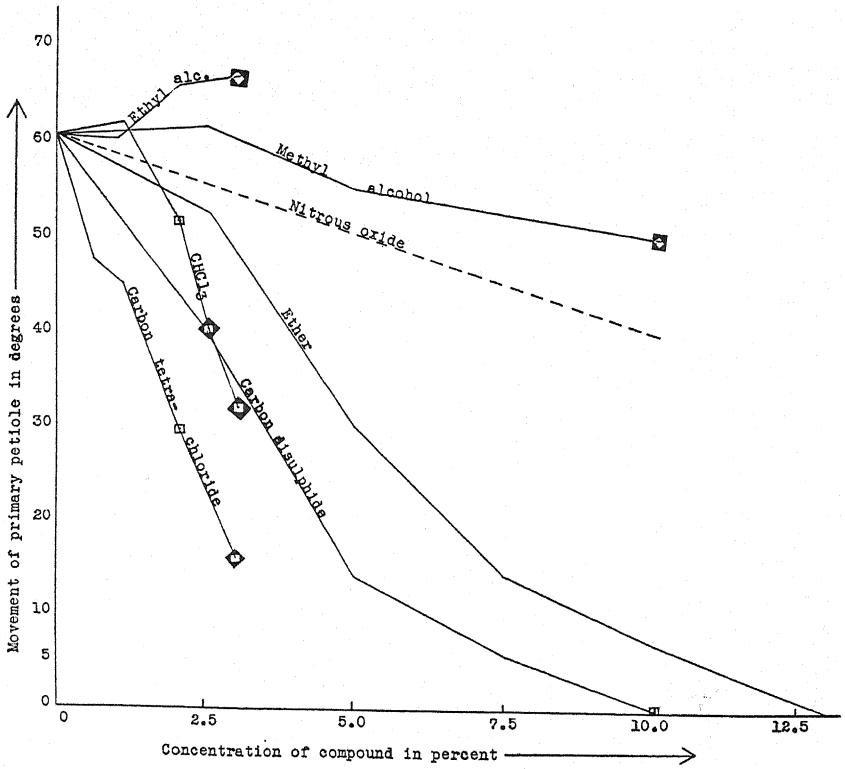


TEXT FIG. 4. The return of sensitivity to the primary petioles of *Mimosa pudica* after having been treated with 15-percent ether vapor for 30 minutes. Data from table 6.

Chloroform

Although various investigators have reported that chloroform will insensitize *Mimosa pudica*, I have been able to bring about this loss of sensitivity only in concentrations that cause serious injury or death. A series of more than thirty experiments testing a wide range of concentrations and exposure times was made between May 1st and September 1st. The preliminary tests in this series established that the physiologically significant range of concentration for chloroform was from less than 1 percent to about 5 percent.

The quantitative data upon the effect of various concentrations of chloroform upon the sensitivity of the primary petiole are given in table 7. The five plants used for each concentration and control were placed in the exposure jars for one-half hour and then removed and their sensitivity determined. As can be seen in text figure 5 those plants in 1 percent



TEXT FIG. 5. The effect of chloroform and certain other compounds upon the sensitivity of the primary petiole of *Mimosa pudica*. Data from table 7. Injurious qualitative effect of the various concentrations of the compounds is indicated as follows: slight injury, open square; serious injury or death, open square within solid one. For ease of illustration, I have arbitrarily assumed 60 to be the angle of movement of the petioles prior to being placed in the presence of the various vapors. Consequently, the values as plotted are slightly different from those given in table 7.

vapor were even more sensitive than the control; those in the 2 percent, although they were about 12 percent less sensitive than the control, also showed permanent injury. The 3 percent chloroform vapor was very injurious, and yet reduced the amount of movement by less than 50 percent. (For appearance of the plants, see Plate XX.)

TABLE 7. *The Effect of Chloroform and Certain Other Compounds upon the Sensitivity of the Primary Petiole of Mimosa pudica*

Date	Temp. (F.)	Compound		Position of Petioles		Angle of Movement
		Name	Percent	Initial	Final	
Aug. 29.....	85	Chloroform	0.0	134.0	73.1	60.9
			1.0	129.8	67.9	61.9
			2.0	142.3	90.7	51.6
			2.5	139.8	99.6	40.3
			3.0	137.8	105.9	31.9
Aug. 30.....	85	Carbon tetrachlorid	0.0	143.3	86.8	56.5
			0.5	151.7	107.6	44.1
			1.0	153.0	111.0	42.0
			2.0	150.4	122.6	27.8
			3.0	146.5	131.0	15.0
Aug. 30.....	85	Carbon disulphid	0.0	143.0	78.5	64.5
			2.5	141.0	96.6	43.1
			5.0	135.3	120.3	15.0
			7.5	121.5	115.7	5.8
			10.0	113.1	113.1	0.0
Oct. 7.....	95	Nitrous Oxid	0.0	126.3	47.3	70.0
			100.0	140.5	96.3	44.2
Sept. 16.....	80	Methyl alcohol	0.0	120.2	50.6	69.6
			2.5	122.5	51.2	71.3
			5.0	123.5	59.3	64.2
			10.0	121.3	63.4	57.9
Sept. 16.....	80	Ethyl alcohol	0.0	116.5	49.9	66.6
			1.0	119.5	53.0	66.5
			2.0	118.3	45.6	72.7
			3.0	120.0	46.7	73.3

The exposure times for the various compounds were as follows: chloroform, 30 minutes; nitrous oxid, 4 hours; carbon tetrachlorid, carbon disulphid, methyl and ethyl alcohol, 10 minutes.

I have reported that very long exposures to ether will often cause a general sluggishness or partial paralysis of the plant which disappears only after several days if at all. This effect is even more pronounced for chloroform. If a plant is exposed to a concentration as low as 1.5 percent for three or four hours the petioles will have become highly elevated and practically insensitive. An elevation of the petioles in chloroform vapor is not usually conspicuous after 30 minutes exposure but does become so an hour or so later as an after effect of the chloroform treatment. An elevation of petiole has been reported by Pfeffer as characteristic for chloroformed *Mimosa* plants and would tend to indicate that the accompanying partial paralysis is his so called anesthesia by chloroform. In the latter part of this paper will be found descriptions and graphs of the effect of various compounds on petiole position.

I have also run tests with concentrations from .25 percent up to 2.5

percent (the approximate lethal concentration) in combination with exposure times varying from a few minutes to twenty-four hours, but so far have found no combination which would cause loss of sensitivity with subsequent complete recovery. A complete loss of sensitivity within 3 to 8 minutes will occur in 4.5 percent vapor, but the leaves always shrivel and die immediately. The leaflets that have been killed by the fumes merely dry up and remain green as though killed by excessive heat. A much longer exposure is necessary to kill the petioles than the leaflets, but I have no data which would indicate that a higher concentration of vapor is required.

Figures 2, 3, 4, and 5 (Pl. XX) show the appearance of plants treated to 2, 2.5, and 3 percent chloroform vapor, respectively, for 30 minutes. Figure 1 shows the appearance of the three plants just before exposure to the vapors. Figure 2 shows the appearance of the plants 30 minutes later on removal from the gas. It will be observed that all leaflets on the plants exposed to 2.5 and 3 percent are entirely closed, and the main petioles have a peculiar drooping near their upper ends as though slightly wilted. The plant in 2 percent appears practically normal. All three sets of pulvini of the three plants at this time were quite sensitive. Two hours after removal the plants had the appearance shown in figure 3. The 2 percent plant seems almost normal with the exception of a slight drooping of the secondary petioles. All leaflets on the 2.5 and 3 percent plants are shriveled up and dry, and the primary petioles are beginning to show the elevation characteristic for chloroform injury. Figures 4 and 5 show the appearance of the plants at the end of 16 and 24 hours, respectively. That the 2 percent plant has been slightly injured is evidenced by the reflexing of the secondary petioles, but all leaves on this plant were retained and remained functional. The 2.5 and 3 percent plants show the usual elevation of petioles and the abscission of the dead leaflets characteristic of chloroform treatment.

Mimosa pudica exhibits a very strong chemonastic response to chloroform vapor. Thus if a plant is placed very carefully in a belljar so that no seismonic reaction occurs, and a slight amount of liquid chloroform added and allowed to evaporate, the secondary leaflets on one or more of the leaflets will suddenly begin to close. After a few minutes all have closed, and not infrequently several of the primary petioles will drop as well. The secondary leaflets may or may not then return to their usual positions depending upon the concentration of vapor, but the primary petioles almost always return to their normal position. This condition is well shown in figure 2 of Plate XX. The two percent plant appears practically normal. The secondary leaflets of the 2.5 and 3 percent plants have failed to open, however, even though the primary petioles have returned to their initial positions.

This chemonastic response must not be confused with that mentioned

by Pfeffer (Ewart's translation, p. 85). He says: "Chemonastic reactions of this kind, like thermonastic responses, are rarely pronounced in character, but the action of chloroform causes a strong curvation in the pulvini of *Mimosa pudica*," and quotes the statement from his Physiologische Untersuchungen (1873, p. 64) as the basis for the statement.[2] It is obvious that this reference does not apply to the closing of leaflets and dropping of petioles which I have described above, but it does seem to describe exactly the elevation of the petioles which I have found to result from exposures to chloroform vapor.

I have attempted, without success, to prevent a chemonastic response in 1 percent chloroform vapor. It seemed possible that if the concentration was increased gradually enough the plant would adjust itself and give no response. Three plants were placed in three separate belljars and a volume of liquid chloroform sufficient to form a vapor concentration of 1 percent placed in each. The chloroform in the first jar was allowed to flow on to the filter paper in the usual manner; that in the second jar was in a large-mouthed, shallow bottle; and that in the third was in a tall vial. The leaflets on the plant in the first jar had all closed within one minute; those in the second, after five minutes; while those in the third just began to close after 15 minutes. I have obtained closure of some leaflets in concentrations of chloroform as low as .1 percent. I might also say that with the exception of ether, nitrous oxid, ethylene, and acetylene, all of the compounds which I have tested induce a chemonastic response similar to that with chloroform.

Carbon Tetrachlorid

Carbon tetrachlorid, as might perhaps be expected from its similar chemical composition, reacts in a manner very similar to that of chloroform. Plants were exposed to concentrations of .5, 1, 2, and 3 percent for ten minutes. As can be seen in text figure 5, it results in no increased sensitivity in the lower concentration. It seems to have a slightly greater repressive action on sensitivity than does chloroform, but even the lethal concentration is not sufficient to reduce it to zero. As has already been stated the plants may show strong chemonastic response to carbon tetrachlorid vapor. The symptoms of serious injury and death are the same as those given above for chloroform.

Carbon Disulphid

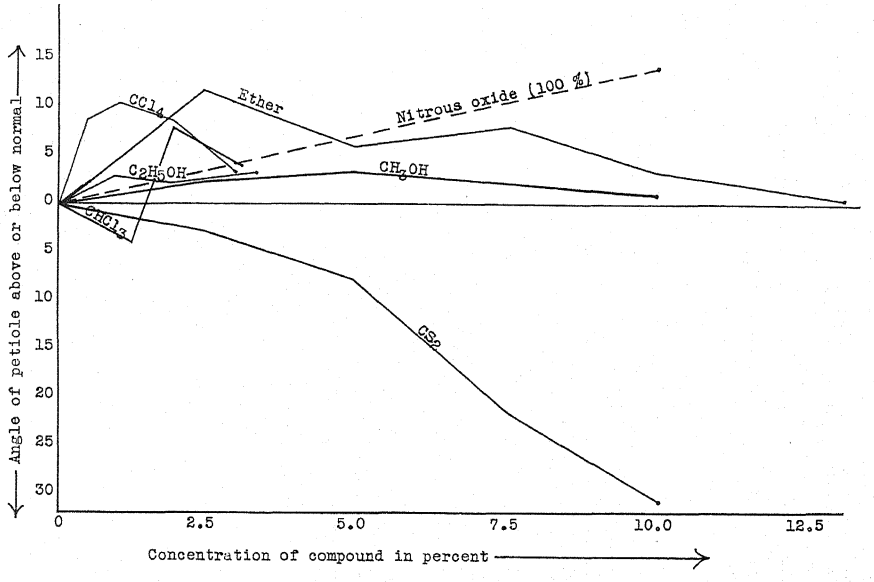
Carbon disulphid is similar in some respects to both ether and chloroform. It will be observed in text figure 5 that the curves for loss of sensitivity in ether and carbon disulphid are very similar indeed, and that the concentrations which induce it in the two cases are only 3 percent different. In

[2] Selbst die Blattstiele, welche sich schon einige Zeit in fester Ruhelage befanden, beginnen eine kleine aufsteigende Bewegung, wenn sich um dieselben, ohne dass Beleuchtung und Temperatur geändert wird, Chloroformdämpfe verbreiten und die Reizbarkeit vernichten. Die beobachtete Erhebung bewegte sich bei verschiedenen Objecten zwischen 5 und 15 graden.

other respects, however, their action is quite different. Thus, as has already been stated, the secondary leaflets become insensitive in ether vapor long before the primary petioles. In carbon disulphid just the reverse is true; that is, the primary petioles become completely insensitive before the leaflets. This is the only compound for which I have found this to be true. Long exposure to 10 percent carbon disulphid affects the leaflets in the same manner as 3 percent chloroform vapor. The lower concentrations are much less toxic. It would seem that the lethal concentration and the concentration required to insensitize are separated by a very narrow margin. The symptoms of injury and death for carbon disulphid are the same as for chloroform and carbon tetrachlorid.

Nitrous Oxid, Ethylene, and Acetylene

Mimosa plants will retain their sensitivity from 1 to 4 hours in approximately 100 percent nitrous oxid and ethylene. The curve for nitrous oxid in text figure 6 shows that plants may remain 60 percent as sensitive in this



TEXT FIG. 6. The effect of chloroform and certain other compounds upon the initial position of the primary petioles of *Mimosa pudica*. Data from table 7.

concentration as in ordinary air. Acetylene, on the other hand, will cause complete loss of sensitivity within 5 to 10 minutes, with no harmful after effects.[3] A slightly reduced concentration (98 percent acetylene and 2 percent oxygen) will make the plants only partially insensitive, however,

[3] The necessity for high temperatures during the experiment to prevent abscission of leaflets and petioles is noted in the paper on sleep movements.

and a slightly lower concentration still (95 percent acetylene and 5 percent oxygen) seems to have very little effect. It has, unfortunately, not been possible to obtain curves for acetylene and ethylene. Some effect of these gases usually caused the plants to respond immediately upon removal from the jars.

The Alcohols

Methyl alcohol is the least toxic of the five alcohols tested and has very little effect in either increasing or decreasing sensitivity. An exposure to 10 percent vapor for ten minutes causes slight injury to the leaflets of the plants, but the sensitivity was only slightly below normal.

The next member of the alcohol series, ethyl, is very toxic to *Mimosa*, a concentration of 3 percent being lethal to the leaflets within ten minutes. The most interesting characteristic of this alcohol is, however, that the sensitivity of the pulvini increases with increased vapor concentration, apparently to the lethal concentration. This is the only compound tested which has shown this effect.

No curves were obtained for propyl, butyl, and amyl alcohols because they vaporize so slowly and are so toxic that serious injury appears after a very few minutes, even before vaporization of the liquid is complete. Qualitative experiments have shown that all three are lethal when present as 1 percent or less.

Alcohol vapors induce a most peculiar chemonastic response in *Mimosa pudica*. I have already reported the closing of leaflets and dropping of petioles which occur in response to chloroform, carbon tetrachlorid, and carbon disulphid. The vapors of the five alcohols tested cause a similar response at first. The petioles then gradually return to their former position and then drop again. This has occurred two and three times in many tests, and not infrequently as many as four times for a given leaf. It would probably respond even oftener were it not for the fact that the plant is seriously injured by this time.

It is not possible to demonstrate this reaction with every trial for it apparently occurs only with very vigorous plants and under optimum conditions of sensitivity. All successful tests were made at temperatures from 30° to 40° C. A concentration of 3 percent ethyl alcohol seemed to induce the response most frequently, but it has also occurred in lethal concentrations of the other alcohols.

It would seem that the killing of cells by the vapors might be the stimulus which induced the response, but if this were true one might expect that other compounds that cause injury, such as chloroform and carbon tetrachlorid, would also cause repeated responses. As these latter compounds, however, unlike the alcohols, have a distinct repressive action on the sensitivity, it is perhaps possible that the failure to obtain similar repeated reactions in the presence of these compounds is due to a dulling of the perception. This is a mere suggestion for the difference in response in the two cases.

Position of Petiole

It was observed during the progress of the qualitative experiments that there tended to be a characteristic petiolar position for some of the compounds tested. Some caused an elevation, some a depression, and others seemed to have little or no effect on the position of the petiole. The greatest elevation occurred in very high concentrations (90 percent or greater) of the three gases, ethylene, acetylene, and nitrous oxid. No quantitative data are available for the ethylene and acetylene for reasons mentioned above, but the petioles of the plants in 100 percent nitrous oxid (text fig. 6) showed an elevation of about 15 degrees above normal. This elevation disappeared in less than an hour after removal of the plants from the jars and is not to be confused with the more or less permanent elevation which results from injury by chloroform and other vapors.

Text figure 6 shows the slight elevation of petioles which occurred in carbon tetrachlorid vapor. It will be observed that the greatest elevation occurred in a concentration of 1 percent. In concentrations greater than this the elevation became less and less until in a concentration of 3 percent, the average petiolar position was only four degrees above that of the control.

I have already quoted Pfeffer's statement that there is an elevation of 5 to 15 degrees in petioles of *Mimosa* in the presence of chloroform vapor. He does not, however, give the concentration at which this elevation occurred. My results indicate that one may find either an elevation or depression depending upon the concentration of vapor used. Thus in text figure 5 it will be seen that at a concentration of 1 percent there was a depression of 5 degrees, while at a concentration of 2 percent there was an elevation of almost ten degrees. The elevation was slightly less in the 2.5 percent and still less in the 3 percent, being then only 3 degrees above normal. The petioles in these two last concentrations became highly elevated subsequent to removal from the jars, as has been described and illustrated in Plate XX.

A low concentration of ether, 2.5 percent, causes an elevation of 12 degrees. The elevation decreased with increased concentration and became approximately zero at the concentration which anesthetizes, 13 percent. The fact that there is no appreciable change in the position of the petiole in concentrations which anesthetize was shown in the following manner. Plants were placed in the belljars and allowed to become adjusted to the new light condition. They were then photographed natural size through the walls of the belljar. The plants were then anesthetized in the usual manner with 15 percent ether. They were then rephotographed, neither the belljar nor the camera having been moved. When one of these negatives was superimposed on the other it was found that there had been no movement. Three successive tests gave the same result.

The two alcohols, methyl and ethyl, upon which quantitative data were obtained seemed to have little effect upon petiole position. The maximum

elevation observed was less than 5 degrees, the average for all points on the two curves (text fig. 5) being slightly less than 3 degrees.

Carbon disulphid was the only compound tested which showed a depression of petioles in all concentrations. The depression was 3 degrees in a vapor concentration of 2.5 percent and became progressively greater (text fig. 5) with increased concentration, reaching a maximum of 33 degrees at 10 percent, the concentration at which sensitivity disappears.

Discussion

Ether first came into general use in surgery about 1848 as a direct result of the successful demonstration of its anesthetic properties by Morton, a dentist of Boston. It had been used almost a decade earlier in the extraction of teeth, but after an unfortunate accident its use had been discontinued. Its use spread very rapidly during the year 1848 and the years immediately following and was soon accepted as good surgical practice.

The first study of the etherization of plants was that of Clemens (1847). He tested the effect of ether on *Berberis vulgaris*, *Mimosa pudica*, etc., and reports complete loss of sensitivity. He does not describe the appearance of the *Mimosa* plant when anesthetized, but does state that two hours are required for the plant to come back to normal.

Leclerc (1853) is the first to give an adequate account of anesthetization of *Mimosa pudica*. He reports as follows:

> I exposed a leaf of *Mimosa* to the vapors of ether for some minutes. It had no effect. I concluded that the experiment had been poorly made. A second plant was placed in a belljar. I surrounded the belljar with sand and under the jar with the plant I placed several vessels full of ether. The sand bed which was rather wide, prevented completely an interchange of air. The experiment was made in sunlight. Ten to twenty minutes later I removed the belljar; all leaflets were greatly extended and the plant was entirely insensitive; the most violent shock, such as acids, fire, or serious mutilation did not produce the slightest movement. One of the leaves was amputated without producing any movement in the rest of the plant. I placed it in my hand; five minutes later, a slight shock caused the leaflets which had been insensitive to move, and a few seconds later all had closed one after the other. The heat of the hand appeared to hasten the return of sensitivity to the amputated leaf. The action of chloroform upon *Mimosa* is more rapid and stronger than that of ether.

This quotation from Leclerc is one of the earliest references to anesthesia in *Mimosa* and summarizes very well the present state of our knowledge. Those investigators (Bert, 1867; Pfeffer, 1873; and Bernard, 1878) who wrote on the subject subsequent to Leclerc, used methods little more quantitative than Leclerc's. No control was kept of the concentration of vapor in the experiments, or at least the concentrations have not been recorded. This is well shown in the following quotation from Pfeffer's Physiologische Untersuchungen (p. 64): "Wenn man ganze Pflanzen oder abgesschnittene Zweige von Mimosen in eine *sehr stark* mit Dampfen von Aether oder Chloroform erfüllte Atmosphäre bringt, so kann die Reizbarkeit

schon innerhalb weinger Minuten sistirt sein." This is also one of the numerous statments in the literature to the effect that both chloroform and ether destroy the sensitivity of *Mimosa*.

There is no doubt that the word anesthesia has been used somewhat loosely, especially in the earlier literature on *Mimosa*. I have used the term to denote complete loss of the power of response with complete recovery after removal from the agent. This is the usual medical definition of the term. It would seem from Leclerc's description (given above) that he considered this complete loss of sensitivity with subsequent complete recovery to be anesthesia. Pfeffer's statement (given above), on the other hand, to the effect that the petioles of *Mimosa* become elevated from 5 to 15 degrees in a concentration of chloroform vapor which destroyed sensitivity, would indicate, as I have already suggested, that he considered the more or less permanent paralysis which follows treatment with chloroform vapor to be anesthesia. That the two reactions are quite distinct is indicated still more definitely by the fact that those concentrations of chloroform which come nearest to insensitizing *Mimosa* in my experiments caused the least elevation of petioles.

A second possibility is that the tests were made with abnormal plants or under conditions of light and temperature not favorable to maximum sensitivity. In this case the amount of movement might be so slight and the rate of movement so slow that it would pass unobserved. I have stated that it was impossible to anesthetize some *Mimosa* plants with ether because, apparently, they were grown under inadequate light conditions. It is just as possible that certain environmental conditions may result in *Mimosa* plants whose sensitivity can be completely destroyed by chloroform, but so far I have never happened to have those conditions. There is no chance of determining the truth of these possibilities because the data presented in the few papers published are too meager.

There seems to be some disagreement as to the appearance of etherized *Mimosa* plants. Leclerc reports that the leaflets are greatly extended. Bert, 1867, states that: "If the plant was at repose it remains with its leaflets expanded, its petioles erect; if on the contrary one has excited it, its leaves remain folded, its petioles lowered (Le Clerc de Tours)." Bert misquotes Leclerc, however, for Leclerc's statement is merely that a *Mimosa* etherized at night remains in the night position and not that it can be anesthetized in the stimulated condition. Pfeffer points out that Bert is undoubtedly wrong in saying that the plant can be anesthetized with its leaflets closed and petioles down as a result of stimulation. I have already stated that it would be perhaps possible to accomplish this if the ether could act in less time than is required for the plant to recover from stimulation.

Bernard (1878) published a drawing showing an etherized *Mimosa* which agrees in all details with those given in my paper on sleep movements. The main petioles, secondary petioles, and secondary leaflets are in the

fully expanded and elevated position such as the plant normally assumes in strong light and favorable temperatures. It would seem that Pfeffer (1873–74),[4] is wrong in his statement that the leaflets on an etherized plant are practically closed, unless the ether he used was contaminated with alcohol.

Herbert (1922) sprayed the leaflets of *Mimosa* with given volumes of liquid chloroform, ether, etc., and obtained responses comparable to those produced by stimulation or wounding; that is, the leaflets closed and the petioles dropped, and then slowly recovered, partially or entirely, depending upon the volume of the liquid which had been sprayed upon them. The experiments are repetitions of the old chemical stimulation tests of De Candolle (1832) with liquid sulfuric and nitric acids, and of Marcet (1848) with liquid chloroform. For some reason he considers his results as evidence that ether and chloroform can not anesthetize *Mimosa*.

Summary II

4. Very careful quantitative experiments made from May 1st to September 1st have shown clearly that of the common animal anesthetics ether is the only one that actually suspended the seismonic sensitivity of *Mimosa pudica* with subsequent complete recovery.

5. Concentrations of ether vapor from 13 to 25 percent (to volume of air) prevented the movement of leaflets and petioles in from 10 to 45 minutes after exposure.

6. The leaflets regained their sensitivity within a few minutes after removal from the ether vapor, but a period of two or more hours was required for the primary petioles to become normal.

7. Chloroform in concentrations of 2.5 percent or greater was lethal and lower concentrations caused injury after long exposures. A concentration of 3 percent chloroform reduced the angle of movement of the primary petiole less than 40 percent.

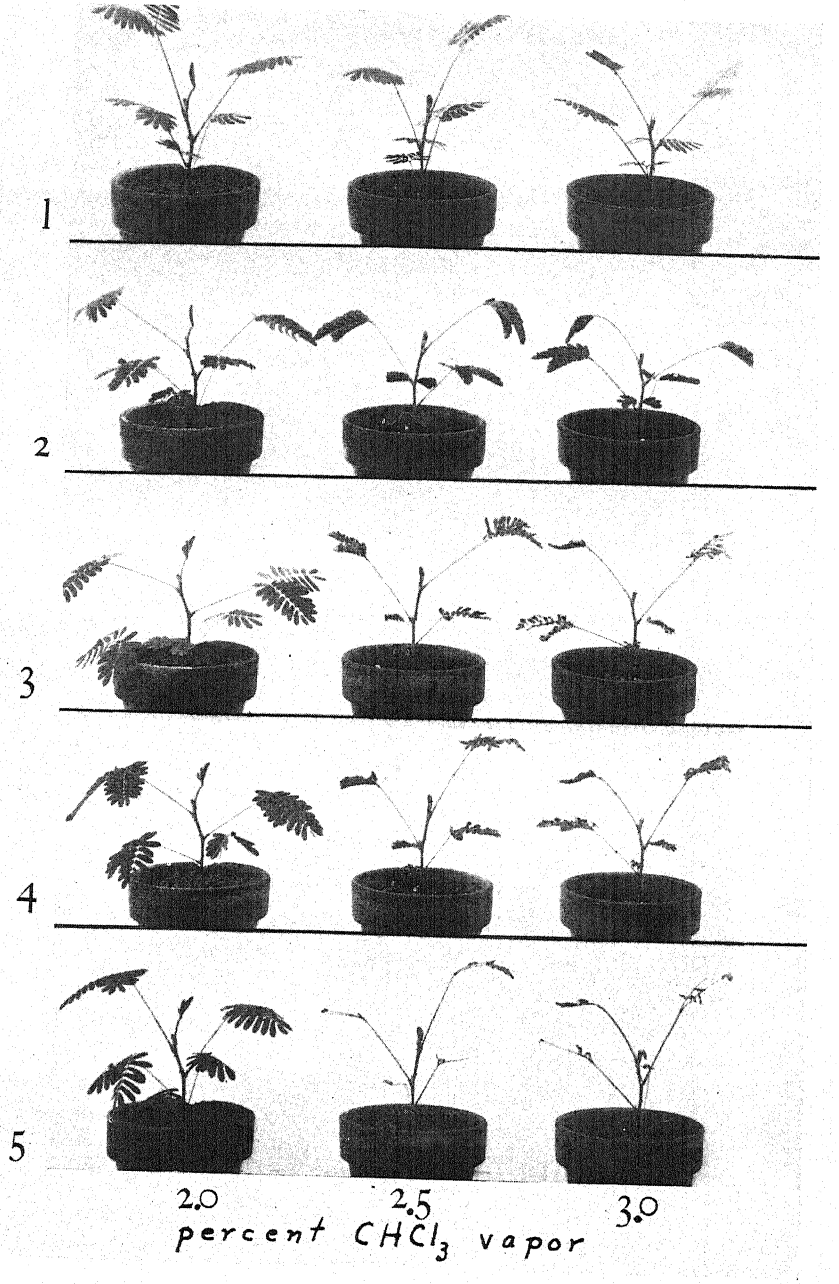
8. Chloroform vapor of a wide range of concentrations induced a strong chemonastic response in the leaflets and petioles of *Mimosa*.

9. Carbon tetrachlorid induced a reaction very similar to that of chloroform. The physiologically significant range of concentration was similar to that of chloroform.

10. *Mimosa* plants retained their sensitivity from 1 to 4 hours in very high (approximately 100 percent) concentrations of nitrous oxid and ethylene.

11. Concentrations of approximately 100 percent acetylene insensitized the plants, while 98 percent made them only partially insensitive, and 95 percent seemed to have little or no effect.

[4] Die Blattchen bleiben beim Aetherisiren nicht ganz eben ausgebreitet, sondern erheben sich etwas, näheren sich übrigens im höchsten Falle bis zu einem Winkel von 45 Grad. (Physiologische Untersuchungen p. 65).



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12. Repeated anesthetization with ether, or repeated exposures to nitrous oxid, ethylene, and acetylene, had no harmful effects on the plants.

13. A 10 percent concentration of carbon disulphid completely insensitized the plants in ten minutes. Longer exposures caused serious injury or death, so carbon disulphid can scarcely be considered as an anesthetic.

14. Methyl and ethyl alcohol seem to have only a slight effect upon sensitivity, the former decreasing it slightly, the latter increasing it slightly.

15. The different compounds tested affected the position of the petiole in various ways. Some caused an elevation, some a depression, and some seemed to be without effect one way or the other.

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EXPLANATION OF PLATE XX

Photographs showing the effect of a thirty minute exposure to 2, 2.5, and 3 percent chloroform vapor.

FIG. 1. The appearance of the three plants just prior to being exposed to the vapors.

FIG. 2. Appearance of the plants thirty minutes later on removal from the vapors. It will be observed that all leaflets on the plants exposed to 2.5 and 3 percent are entirely closed, and the main petioles have a peculiar drooping near their upper ends as though slightly wilted. The plant in the 2 percent appears practically normal. All three plants were quite sensitive at this time.

FIG. 3. Appearance of the three plants two hours after removal from the vapors. All leaflets on the 2.5 and 3 percent plants are shriveled up and dry, and the primary petioles are beginning to show the elevation characteristic of chloroform injury.

FIGS. 4, 5. Appearance of the plants 16 and 24 hours after removal from the vapors. That the plant in 2 percent vapor has been injured is evidenced by the reflexing of the secondary petioles, but all leaves on this plant were retained and remained functional. The 2.5 and 3 percent plants show the usual elevation of petioles and the abscission of the dead leaflets characteristic of chloroform treatment. Compare the qualitative effects shown in these figures with those recorded in text figure 5.

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PHYLOGENETIC EVOLUTION OF THE PHYLLOME

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It is hardly possible to give in a short paper a convincing account of a theory which must be based on an extensive array of facts, only a part of which (1) have thus far been presented.

The problem has lately been much discussed from the ontogenetic point of view (see the exhaustive bibliography in Foster, 7). The morphological and phylogenetic standpoints, however, have been very much neglected. The ontogenetic investigations partly support my theory but more often contradict it. This, however, is of no consequence, as a morphological problem must be solved primarily by comparative morphological investigations as well as by the evolutionary history of plant organs. Even Glück (9, p. 637–638), a prominent member of Goebel's school, correctly pointed out that ontogenetic facts do not always agree with the true morphological character of the organ. Thus he mentions that the stamens of some plants (*Euphorbia*, *Casuarina*, *Najas*, *Typha*, *Cyclanthera*) though they clearly belong ontogenetically to the axis, are undoubtedly metamorphosed phyllomes. Many other cases (see Velenovský, 11) of a similar nature have been emphasized, and Schrödinger (10, p. 185–186) points out that in not less than five genera of the Cunoniaceae the stipules arise "nachweislich echt freiachsenbürtig, d.h. aus selbständigen Primordien, für deren Vorwölbung im Innern des Sprossgipfels vollkommen selbständige Zellvermehrungsherde differenziert werden." Hardly any botanist, however, would assume that they do not belong to the respective leaves but that they are *organa sui generis*.

It is hardly necessary to repeat in detail my theory of the phylogenetic differentiation of the phyllome according to which the most primitive form is a one-membered, broadly inserted leaf which in further evolution differentiated into a lower member (the sheath) as the predominantly mechanical organ and the blade as an organ designed for assimilation. In further evolution the auricles of the sheath, which are of rather common occurrence, lengthen while the sheathing part shortens (*stipulae adnatae*, called by Glück more appropriately *stipulae vaginales*); finally, after total disappearance of

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the sheathing base, we find these enlarged auricles perfectly separated as free stipules.

This theory which I tried to bring in agreement with the doctrine of anaphytosis (Domin 1, 2, 3, 4) is essentially identical with that of Tyler (1897) and various other authors but was rejected as inconsistent and speculative by Foster (7, p. 153) who bases his ideas on ontogenetic investigations. Schrödinger (10) published an exhaustive criticism of my theory and states (l. c., p. 176) that „ alles in allem genommen, wird man Domins Theorie—soweit es sich um Monokotyle handelt—die Anerkennung nicht versagen können." As regards the dicotyledons he opposes my theory but finally adopts practically the same view since he states (p. 191–192):

> Der Entwicklungsgang, den die stipulaten Scheiden ontogenetisch einhalten, stimmt—soweit die bisherigen Erfahrungen reichen—regelmässig mit jenem überein, den wir ihnen phylogenetisch zumuten müssen. Es werden zuerst reine Scheiden angelegt und diesen wachsen dann sekundär Stipeln zu. Auch für die grundständigen Stipeln ist eine Reihe von Fällen bekannt, wo der ontogenetische Entwicklungsgang mit dem vermuteten phylogenetischen übereinstimmt, weil die Stipeln als Aussprossungen aus Scheiden entstehen und erst sekundär—durch nachträgliche Hemmung des Scheidenwachstums—zu grundständiger Stellung gelangen.

It is evident that the phyllome is an abstract conception represented in living plants by all possible shapes and forms into which the leaf primordium was moulded. Instances where a sharp limit between the phyllome and caulome vanishes are extremely rare (*Utricularia*, see Goebel and Glück) and can be only explained by secondary metamorphosis.

When studying the evolution of the leaf in the most primitive vascular plants, viz. Pteridophyta (5), I found that it is impossible to maintain the doctrine of anaphytosis as valid. The phylloids of bryophytes, belonging to the *x*-generation and lacking true vascular bundles, are analogous but not homologous with true leaves of the sporophyte (*2x*-generation) of the vascular plants. The fossil Devonian Psilophytales show in a rather convincing manner that the leaves developed as emergences of the axis; into these emergences the vascular bundles entered and gradually the leaves became independent lateral organs, morphologically and anatomically different from the axis. The Rhyniaceae (including the Horneaceae and Pseudosporochnaceae) are the most primitive types with leafless, apparently assimilating, and hemiblastically branched stems. In Psilophytaceae we observe a type with small spine-like emergences on the stems, representing a kind of imperfectly differentiated leaves, whereas the Asteroxylaceae show branches with dense, small simple leaves of the lycopodioid character. Also the axis and leaves of the Carbonian *Stauropteris* (Coenopteridales) are not yet strictly differentiated; the leaves here are "branched" in various planes, the anatomical structure of the leaf-rachis is identical with that of the axis and no flat blade is formed. These paleozoic types, in the former case leafless or microphyllous, in the latter macrophyllous, seem to indicate

that the leaves are organs differentiated from the emergences of the axis and in further evolution specialized anatomically and morphologically.

With respect to these primitive fossil Pteridophyta I feel obliged to abandon the doctrine of anaphytosis. My theory of the phylogenetic evolution of the phyllome is, however, not necessarily connected with the doctrine of anaphytosis and notwithstanding the objections raised against it and the rejection of it as an idealistic speculation, I find it even at present a theory which explains quite satisfactorily all the known facts. The ontogenetic objections cannot be looked upon as decisive as they are chiefly dependent on the evolution of the multipotent leaf primordia.

Phylogenetically it is possible to distinguish the following stages in the evolution of the phyllome:

1. Emergences of the axis as the first step towards a differentiation of a phyllome as a distinct organ (known only in the Devonian Psilophytaceae).
2. One-membered, undifferentiated, assimilating leaf, inserted usually with a broad base.
3. Indistinctly two-membered leaf beginning to differentiate according to its two principal functions, into an upper part (blade) designed for assimilation, and into a lower part (sheath) as a mechanical organ.
4. Two-membered leaves consisting of distinctly differentiated blade and sheath (without auricles), sometimes separated by a petiole.
5. Leaves similar but with auricles on the sheath, the auricles taking over the function of the sheath.
6. Similar leaves with shortened sheaths (*stipulae adnatae* or *vaginales*).
7. The auricles of the sheath become independent and form free stipules.
8. The free stipules are modified in various ways (in form, size, and position).

According to this theory the *stipulae vaginales* are phylogenetically an older type than the free stipules which are morphologically homologous with sheath-auricles but separated from the leaf due to the total disappearance of the sheath. Contrary to this view, some prominent authors (for instance Velenovský and Hugo Glück) regard free stipules as an original structure and a starting point for the sheath which can be brought about by their enlargment and fusion. Eichler (1861) states that ontogenetically the stipules are the more original structure the more they are inserted towards the leaf base; free stipules are therefore in his view the most original condition and the auricled sheaths the most derived.

The differentiation of the phyllome, as above outlined, follows along certain ecological lines, but is exceedingly complicated and shows progressive as well as retrogressive developmental steps so that leaves of the same type are often phylogenetically not the same. Two-membered leaves—even if the lower member is represented only by free stipules—can by abortion of the stipules (or the sheath) become simple but the cataphylls show sometimes in such cases their true value.

In primary leaves and in cataphylls the lower leaf-member (the sheath) often makes its appearance even in cases where the assimilating leaves are without a sheath. Thereby the cataphylls repeat in a certain sense the phylogenetic evolution, or more accurately said, they show in full development the lower member, suppressed or reduced in the foliage leaves. This idealistic viewpoint with its "recapitulatory ideas" was the principal objection against my and other similar theories. I therefore formulate anew my views as follows:

During phylogenetic evolution, the originally simple phyllome became two-membered in accordance with its two principal functions. Stipules belong in all cases to the sheath; however, after the disappearance of the sheath, they may become independent and free and even change their position. The suppressed sheath, however, reappears not rarely in cataphylls and even if this may be physiologically explained by a complete reversal of the growth rates existing between the two members, this structural development brings about the return of the phylogenetically older stage. The repetition of the supposed phylogenetic evolution, shown often in the cataphylls, is in reality not an atavistic evolutionary recapitulation, but caused primarily physiologically. Nevertheless, the functional change brings about structures not manifesting themselves in the mature assimilating leaves but which structures we suppose to have been existent during the phylogenetic differentiation of this plant organ.

The above scheme shows the gradual evolution of a two-membered phyllome. Once this stage was reached, the further metamorphosis followed along the most diverse progressive and retrogressive lines, now leading to a potential or absolute suppression of one member, then again to a fusion of both members, to a differentiation of a petiole from the one or the other member, and to many other modifications, often corresponding to a functional change.

Rather often we observe in the cataphylls a series of structures recapitulating the supposed phylogenetic differentiation of the phyllome, but here also may occur a reversion of structures, as for instance Shull (1905) has shown in *Sium cicutaefolium*.

The recapitulation as exhibited in the cataphylls, is, however, not quite a true one, since it usually starts with the suppressed lower member, with or without a rudiment of the upper member, whereas the phylogenetic series begins with a simple, non-differentiated leaf structure.

How many-sided the evolution of the phyllome may be is shown most clearly by the dicotyledons, but even in the Pteridophyta we observe many developmental lines, some complicated and with many side-lines, while others again are consistent throughout. The lycopodioid type, for instance, differs essentially from the macrophyllous Filicales, (see Domin 1, p. 137), and that to such a degree that Lignier declares the leaves of Lycopodineae as homologous structures with the phylloids of bryophytes.

The leaves of the monocotyledons represent phylogenetically older stages in the leaf evolution and are rather uniform, varying from simple and broadly inserted forms to leaves differentiated into a sheath (with or without either ligule or auricles) and a usually sessile blade. The leaf evolution here did not reach the stage of the "stipulate sheaths" or even of the free stipules (rare exceptions occur in the Helobieae); this is, of course, an important character of the whole group.

The leaves of dicotyledons show all possible developmental stages, although more advanced types predominate. Leaves without any stipular organs are either two-membered leaves with the lower member aborted or sometimes also one-membered leaves. Their stipules, when present, can change their position and instead of being lateral (Glück's *stipulae liberae*) they become axillary, interpetiolar, or antidromous; they are either free or divided, or they are fused in various ways (ocrea, ligule); as an exception (for instance in *Hydrocotyle*) they embrace the petiole from outside. We find them in the most surprising variations in many families, in some constantly, in others often or occasionally. Thus the following families show regularly, or only in some genera, stipular organs of various structures: Aquifoliaceae, Araliaceae, Begoniaceae, Berberidaceae, Betulaceae, Bombacaceae, Capparidaceae, Caryocaraceae, Celastraceae, Chlaenaceae, Cistaceae, Cunoniaceae, Dichapetalaceae, Droseraceae, Elaeocarpaceae, Elatinaceae, Erythroxylaceae, Euphorbiaceae, Fagaceae, Flacourtiaceae, Geraniaceae, Hamamelidaceae, Leguminosae, Loasaceae, Lythraceae, Magnoliaceae, Malpighiaceae, Malvaceae, Melianthaceae, Moraceae, Myrothamnaceae, Ochnaceae, Onagraceae, Passifloraceae, Penaeaceae, Piperaceae, Platanaceae, Portulacaceae, Ranunculaceae, Rhizophoraceae, Rosaceae, Rubiaceae, Salicaceae, Saxifragaceae, Stachyuraceae, Staphyleaceae, Sterculiaceae, Tiliaceae, Trigoniaceae, Umbelliferae, Urticaceae, Vitaceae, Vochysiaceae, Zygophyllaceae, etc.

The metamorphosis of the stipular organs of dicotyledons is really amazing and is partly structural, partly functional. A correlation of the growth rates of blade and stipules is generally known and was also demonstrated experimentally (Goebel, Kronfeld, and others). A very interesting phenomenon is the so-called *coleophylly* which I described and pictured (5) in *Ranunculus bulbosus* where it occurs almost normally. Also chorisis of stipules, both by cleavage and deduplication, is well-known in some cases. In addition we know, of course, assimilating or spiny stipules as well as stipules transformed into glands or hairs, and many other modifications which cannot be recorded in this short contribution.

In some dicotyledons the stipules, phylogenetically a part of the lower leaf-member, acquired a complete independence ontogenetically as well as in their position and structural evolution, so that without an exhaustive comparative study one would feel inclined to look upon them as on organs quite independent of the leaf. Their many-sided and most interesting modi-

fications in the floral region were described with great accuracy by Hugo Glück (9).

Twenty years ago I prepared for publication my studies on the stipular organs of the monocotyledons which formed only a smaller part of my investigations made chiefly in Buitenzorg, Java. I hope that my results on dicotyledons, when published, will show that the theory I have established holds good for all vascular plants, even though the structural evolution of the dicotyledon leaf is much more complicated than that of the monocotyledons. At the same time I shall have an opportunity to discuss various objections and theories published in the last two decades.

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PHYLOGENETIC AND ONTOGENETIC INTERPRETATIONS OF THE CATAPHYLL[1]

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To many of the early students of plant morphology it seemed only natural to suppose that the organization and position of cataphylls in the shoot system indicated their derivation from foliage leaves in accordance with the need for protective organs about the bud during certain parts of the year. This assumption seemed to be well supported by the frequent occurrence of leaves intermediate in character between foliage leaves and cataphylls, a phenomenon which even at the present time is cited, especially in botanical texts, as showing that scales in reality are "reduced" foliage leaves. Such formal considerations, while of some pedagogical value, entirely neglect the factors responsible for the striking divergence between the scale and the foliage leaf and thereby give to the problem a static quality which has done much to discourage active investigation. Indeed, the problem of the successive and periodic development of scales and foliage leaves on the shoot is still in nearly the same stage as it was during the time of Goethe (13), Alexander Braun (1), and De Candolle (3).

The causes for this unsatisfactory state of knowledge have been examined in a recent paper by the writer (8) and the conclusion reached that at present we have no satisfactory morphological or "causal" interpretation of the cataphyll. This statement was made with due regard to the "transformation theory" developed by Goebel (11, 12) and to the various phylogenetic theories of the scale as given by Velenovský (18), Tyler (17), Domin (6, 7) and Cook (4). However, at the 5th International Botanical Congress, Domin, in a re-statement of his theory of the evolution of the phyllome,[2] maintained in fundamentals his previous view of the nature of the cataphyll and questioned the value of ontogeny as a method of approach to the problem of foliar morphology. It is not my purpose to analyse in detail Professor Domin's views as to leaf evolution but in the following discussion an effort will be made to show that until our knowledge of the nature of the primitive leaf in seed plants has been *inductively* established, the problem of cataphyll morphology can be most effectively approached by intensive developmental studies. A few facts will serve to indicate the uncertain position at the present time of phyletic theories of the scale.

[1] Contribution from the Botanical Laboratories of the University of Oklahoma, N. S. No. 7.

[2] Professor Domin's re-stated theory appears in the present issue of the Journal, pp. 237–242. Unless otherwise noted, reference is made to this paper.

It seems almost unnecessary to state in these days when all phylogenetic systems are experiencing revision and re-examination in the light of new facts that we are very far from an understanding of the details of foliar evolution.[3] The occurrence of well-developed cataphylls in such an archaic seed plant as *Ginkgo biloba* L. and the wide-spread distribution of bud scales in both temperate and tropical angiospermous families (Foster 8, p. 124–126), suggest that the "scale habit" is very ancient. Parallel and independent evolution of cataphylls in various groups of the seed plants, as well as complex anatomical specializations of these organs, have occurred but it is only in keeping with the facts to state that the details of this complicated phyletic development are as yet completely obscure (Foster 8, p. 137–146, 152–156).

In spite of such difficulties in the way of a securely-founded phylogeny of the leaf, Domin advances the following theory. The most primitive leaf was a simple, broad-based assimilating organ which in the course of evolution gradually differentiated into (1) a lower protective "member" or sheath (frequently represented in cataphylls) from which free stipules have evolved by a gradual reduction of the sheathing base and (2) an upper assimilatory "member" or blade. Domin recognizes seven stages in such an evolutionary development which he will presumably illustrate by examples from dicotyledons in his forthcoming paper on the stipular organs in this group. Upon what consideration does Domin's theory rest?

In his original paper on stipular formations in monocotyledons (6, p. 149) Domin based his notion of the primitive leaf upon the "Doctrine of Anaphytosis" according to which "the leaf represents the upper lateral free end of an anaphyte and consequently it must, in its most primitive form, surround the whole periphery of the stem with a broad base." Although Domin maintained that such an assumption is supported by the frequent sheathing bases of the primary leaves of seedlings, Schrödinger (15, p. 180) states in his critical analysis of Domin's theory that no case exists in dicotyledons where broadly inserted primary leaves occur in connection with plants possessing simply petiolate foliage leaves. Domin however, in the re-statement of his theory, now abandons the doctrine of anaphytosis as a result of his studies of certain fossil Pteridophyta but nevertheless states that his theory, which I had previously characterized as speculative and inconsistent (Foster 8, p. 152–153), "explains quite satisfactorily all the known facts."

The facts which Domin's theory attempts to interpret were obtained by a broad survey of adult foliar organization. The value of this work cannot be denied and even Schrödinger (15, p. 175–176) who has critically examined them in detail agrees that Domin has shown that the monocotyledons have been derived from an angiospermous stock in which leaves with sheathing

[3] Cf. Campbell (2, 317–319) who has recently drawn attention to the difficulties in the way of a phyletic interpretation of the monocotyledonous leaf.

bases were typical and that in this group the absence of a sheathing base (e.g. *Dioscorea*, *Tamus*) is a secondary phenomenon. Schrödinger, however, rejects Domin's theory as being generally valid for the angiosperms as a whole. This point is worthy of note since Domin, in the re-statement of his theory, gives the impression that Schrödinger adopts a viewpoint similar to his own. This is an error, for a careful examination of Schrödinger's paper will show that while his ontogenetic studies led him to nearly the same conclusion as Domin as regards the phylogenetic derivation of stipules from the sheath, these comparative developmental studies and conclusions were *definitely confined* to genera possessing stipulate or sheathing foliage leaves. Domin, however, attempts to apply his theory of the two-membered leaf even to the very numerous dicotyledonous families which are both sheathless and exstipulate. In such instances Domin supposes that the lower member has either been completely lost in evolution or else that it has given rise to the petiole. The considerations which lead Domin to this hypothesis are derived from his views as to the morphology of cataphylls and require careful examination.

It is obvious that Domin, in his original paper, regarded the organization of cataphylls as strong evidence in favor of his theory of the evolution of the two-membered leaf. Writing of *Ginkgo biloba* L. he says (6, p. 131) that the typical leaf sheath appears only in the primary leaves, bud scales, and transitional leaves while it appears "reduced" in the foliage leaves to a normal petiole. He further states that "analogous atavistic leaf forms" occur in the primary leaves or cataphylls of such genera as *Aesculus*, *Acer*, *Cornus*, and *Viburnum*. In his later paper Domin (7, p. 18) attempts to extend his "recapitulatory interpretation" of cataphylls to a number of dicotyledonous families, viz.: Ericaceae, Saxifragaceae, Cornaceae, Caprifoliaceae, Aceraceae, Sapindaceae, Juglandaceae. In these families he maintains that one finds the lower member (i.e. the sheath) in its "original form" in the cataphylls which are thus considered to have given rise to the petiole of the foliage leaf. In view of the recapitulatory value ascribed by Domin in earlier papers to cataphylls, one is rather confused by his position in this regard as set forth in the re-statement of his theory. He says: "In primary leaves and in cataphylls the lower leaf-member (the sheath) often makes its appearance even in cases where the assimilating leaves are without a sheath. Thereby the cataphylls repeat in a certain sense the phylogenetic evolution, or more accurately said, they show in full development the lower member, suppressed or reduced in the foliage leaves." This quotation would be taken to show that Domin adheres to his original views. Yet in the next paragraph he states: "The repetition of the supposed phylogenetic evolution, shown often in the cataphylls, is in reality not an atavistic evolutionary recapitulation, but caused primarily physiologically. Nevertheless, the functional change brings about structures not manifesting themselves in the mature assimilating leaves but which structures we suppose to

have been existent during the phylogenetic differentiation of this plant organ." This quotation appears to indicate that although Domin recognizes the relation of physiological-developmental conditions to scale conformation, he nevertheless prefers to regard the cataphyll morphologically as typifying an earlier type of foliar organization in many cases.

Such reasoning seems of little value to the writer since it forces us to make assumptions regarding the cataphyll which, as I have attempted to show, appear quite beyond the realm of scientific investigation at the present time. Schrödinger is very emphatic in respect to Domin's view of the cataphyll for he states (15, p. 178) that "to regard cataphylls as the ancestral form of the foliage leaf is under all circumstances an error." Cases do occur, for example in certain members of the Rosaceae (Domin 7, p. 12–15), where the cataphylls are sheath-like in structure although the foliage leaves possess paired basal stipules. Such examples, when more fully investigated, especially from an ontogenetic standpoint, may shed additional light upon the vexed question of stipule morphology (Schrödinger 15, p. 178) but they seem to bear little relationship to the problem of cataphyll morphology in groups possessing exstipulate and sheathless foliage leaves. For example, *Acer*, *Fraxinus*, *Cornus*, *Juglans*, *Aesculus*, all belong to families characterized by the absence of stipules and if, as Domin has admitted, the form of cataphylls is *not* primarily an atavistic phenomenon it is surely begging the question to interpret the scales in such instances as representing structures we "suppose" to have existed during leaf evolution.

Within recent years, a certain amount of new experimental and developmental data have accumulated which seem to shed new light upon the problem of cataphyll morphology. These studies in the first place have failed to support Goebel's (11, 12) "transformation" theory according to which cataphylls are to be regarded as arrested formations (Hemmungsbildungen) of foliage-leaf primordia which at some arbitrary developmental stage have experienced an arrest in their segmentation and have then pursued a divergent ontogeny leading to the enlarged and modified development of, for example, the leaf-base or the stipules.[4] As strong evidence in support of his theory, Goebel (11) has cited the results of defoliation experiments which he believes demonstrated that primordia, which by their position on the axis would normally have been transformed to bud scales, were able under the changed conditions to develop to transitional forms or even to foliage leaves. Schüepp (16, p. 795), however, in some recent defoliation experiments on *Acer pseudoplatanus* L. concludes that Goebel's experiments (conducted largely on *Prunus Padus* L.) merely show that under certain conditions foliage leaves may replace bud scales but that they fail to prove that microscopically visible primordia at a relatively high stage of segmentation may become either "typical" foliage leaves or "typical"

[4] For a detailed critique of Goebel's theory cf. Foster (8, pp. 146–149) and Schüepp (16, pp. 794–798).

scales. On the contrary, Schüepp's experiments show that a foliar primordium may be determinate (in the morphogenetic sense) at the very earliest period in ontogeny, even before segmentation into "leaf-base" and "lamina" has occurred. Thus the presence of a laminar primordium in connection with a cataphyll constitutes no proof that foliage-leaf characteristics early dominate in the primordium of such an organ. My own studies (10) on *Aesculus Hippocastanum* L. support this view, especially the data obtained on the difference in the ratio of growth rates between lamina and hypopodium in the scale and in the foliage-leaf primordium.

The recent work of Diels (5) on the cataphylls and foliage leaves of *Asarum europaeum* are particularly significant in regard to Goebel's theory since his defoliation experiments seem to show that in this plant "scale determination" occurs at a period when it is virtually impossible to recognize "foliage-leaf qualities" in the primordium. Until the gulf between the chromosomal transmission of hereditary characters and their expression in the adult organism has been spanned it would seem that any ontogenetic theory of leaf metamorphosis can have only an abstract significance (Klebs 14, p. 4–6) and that while a foliar primordium may be theoretically multipotent, experience seems to show that it becomes determinate at a very early stage in ontogeny (Schüepp 16, p. 788).

In view of the strictly limited amount of new data at hand, it would be premature to attempt to frame a developmental "theory" of the scale at present. Nevertheless, certain facets of the problems, which can and are being studied by the ontogenetic method, seem to represent fundamentals and as such are worth summarizing in the present paper.

First of all, ontogenetic studies have shown the prime importance of following the *complete* history of development of a cataphyll from the time of its appearance as a meristematic protuberance at the growing point until its mature form and size have been attained. The writer (10) attempted such a detailed study on the cataphylls of *Aesculus Hippocastanum* L. and as a result was able to offer an analysis, in terms of rates and type of growth, of the form changes exhibited by the successive scales of the bud, as well as of the adult divergence between scale and foliage leaf in this species. Schüepp (16) has made a similar type of study in connection with *Acer Pseudoplatanus* L. and his general results, especially as regards the growth factors involved in the divergent ontogeny of scale and foliage leaf, are in close agreement with the situation in *Aesculus*. Further investigations along these lines may be expected to shed light upon the fundamentally simple form of the cataphyll and especially upon the problem of growth-correlations in different parts of a developing foliar organ.[5]

Secondly, an analysis of the internal differentiation of the scale, especially

[5] Favorable material for such studies is offered especially by members of the Fagales where certain genera e. g. *Castanea*, possess unsegmented cataphylls although the foliage leaves are stipulate. For further details regarding bud-structure in this group cf. Foster (9).

from the standpoint of the position in it of meristematic tissue and its duration, presents a field replete with promise for the experimental morphologist. Schüepp (16, p. 800), for example, has shown clearly how the type and position of meristematic tissue and the rapidity of tissue maturation are important "causal" factors in determining whether a foliar primordium in *Acer Pseudoplatanus* L. develops into a cataphyll or into a foliage leaf. In a paper read before the 5th International Botanical Congress, I suggested that scale-development in certain cases may be characterized by rapid vacuolization in the foliar primordium and the restriction of typical meristematic tissue to its margins. This marginal growth, which Schüepp assigns in the case of *Acer* to a "Randmeristem," coupled with the absence of growth in thickness and the reduction or complete suppression of leaflet primordia, seem to account for the vaginate form of the cataphylls in *Acer Pseudoplatanus* L., *Aesculus Hippocastanum* L. and *Carya*. Whether these particular cases represent a general condition can only be determined by further investigation.

Thirdly, developmental studies have emphasized the importance of the periodic alternation of cataphylls and foliage leaves on the shoot. Two aspects of this phenomenon seem of particular importance. First, the fact, as shown by the studies on bud development from the laboratories of Blaauw,[6] that in perennial plants we have to deal with fairly definite scale- and foliage-leaf-forming periods. I have already drawn attention to the importance of this fact in the case of *Aesculus Hippocastanum* L. and have suggested (10, p. 489–492) that the two types of "transitional forms" developed in this species reflect changing conditions in the physiology of the shoot which control, in some way, the periodic alternation of bud scales and foliage leaves. Secondly, an analysis of Blaauw's work by Schüepp (16, p. 792–793) indicates that the bud scales in a number of cases are laid down at the growing point at a faster "tempo" than are the foliage leaves in the same species. As Schüepp points out, this fact is strongly opposed to the assumption that scale formation is due to an arrest in growth (Wachstumshemmung). At any event, a closer study of this phenomenon is needed and may open up a new aspect of the problem of the repetitive development of foliar organs at the growing point.

Although ontogenetic study is insufficient by itself to explain all the involved aspects of the problem of cataphyll organization, this line of attack promises to contribute much to our understanding of these organs if it is consistently applied to a large number of cases. When the nature of the primitive leaf in angiosperms and the details of its evolution have been inductively established, a phylogenetic interpretation of the cataphyll may be feasible. Until that time, we must rest content with the concrete evidence furnished by ontogeny and experimentation.

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[6] Cf. Foster (8, p. 124, footnote 1) for specific literature references.

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POLYPLOIDY IN *PETUNIA*

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INTRODUCTION

The author's work on *Petunia* was started chiefly to study cytologically the behavior of triploids. The present paper presents the results of a study of triploidy and of some other phases related to this main problem. The races of plants used in this experiment were obtained at the University of Maine and the preliminary crosses were made there in the spring of 1929. The main part of the work, however, has been done at the Bussey Institution, Harvard University, since the fall of 1929.

In *Petunia* the diploid has $n = 7$ chromosomes. The ordinary diploid has small flowers and narrow calyx. Among a group of such plants appeared one with very large flowers and vegetative parts which proved upon examination of PMC (pollen mother cells) to be tetraploid. A second strain of diploids had flowers of a very deep purple color, deeply lobed and slightly ruffled at the edge, of a size intermediate between the small diploid and the tetraploid, and had broad sepals. A third diploid strain, appearing as a bud mutation from the large flowered diploid, had flowers a little smaller than the parent plant and, like the ordinary diploid, lacked the deeply lobed and ruffled character and had narrow sepals.

The large flowered diploid was found to be heterozygous for the size factor giving approximately a ratio of 1 large to 1 small when crossed with ordinary small flowered diploids. When the flowers of the mutated branch were selfed they bred true. The small segregates from the cross and the progeny of the mutated branch were identical in almost all respects.

In order to simplify the recording of differences, the following abbreviations are used throughout this paper: 14*L*, large flowered tetraploid; 14*S*, small flowered tetraploid (obtained from selfing 14*L*); 7*L*, large flowered diploid; 7*S*, small flowered diploid; 7*Lnc*, large flowered diploid with narrow calyx (bud mutation).

Numerals at the right of an abbreviation (e.g. $7S_3$, $14L_4$, etc.) indicate the number of the plant of the strain given.

The tetraploid was selfed and from a single capsule a hundred or more seeds were germinated but only 7 plants were reared, 5 having large flowers and calyx with wide sepals and 2 having small flowers and small calyx with narrow sepals. Later 31 plants were grown, 22 of which were large flowered (the size of calyx and other parts always correlated with flower size) and 9 small flowered. Obviously, then, the original tetraploid plant was hetero-

zygous for the factor for flower size and, since the diploid was pure for the small flowered character, the assumption seems to be justified that, in addition to the doubling of chromosomes, a point mutation had occurred that affected size of flower and other parts of the plant. The $7S$ plants showed a variety of variegation patterns; when selfed they bred true to all main characters except the variegation patterns. The progeny of $14L$ showed all the grades of variegation found in $7S$ and were also variable in many minor points. For example, among 36 tetraploid plants, 8 were sickly looking and of these one was found to have 29 somatic chromosomes instead of 28.

Plants $7S$ and the original $14L$ were crossed reciprocally with apparent success in both ways.

In the fall of 1929 various pedigreed seeds were planted in the greenhouse of the Bussey Institution but the crosses $7S \times 14L$ and $14L \times 7S$ showed no sign of germination even after some weeks, while other crosses had seeds germinating in less than five days. When seeds from these crosses which had not been planted were examined it was found that the seeds of those large seed capsules were empty, though occasionally there were discovered out of about 200 seeds in a capsule one or a very few seeds that were full. The several hundred seeds that were left were all planted and finally four seedlings were successful in coming to maturity. These four plants were from the cross $7S \times 14L$, three of them large and one small flowered. Some seedlings broke through the seed coats but did not grow any further. Later more plants were obtained from cuttings.

In the spring of 1930 more triploids were obtained from $14S \times 7L_6$ and $14S_{2-28} \times 7S_6$. One plant from this last cross had 20 chromosomes.

The four triploids first obtained were back-crossed reciprocally to various diploids and tetraploids. The results are given in table 1. The chromosome counts of these progeny were made from root tip sections.

TABLE 1. *Chromosome Numbers of Progenies from Back-crossed and Selfed Plants*

Cross	No. of Somatic Chromosomes in Progeny						
	14	15	26	27	28	35	Total
Diploid × Triploid	39	12					51
Triploid × Diploid		No progeny					
Triploid × Tetraploid				11	2	1	14
Tetraploid × Triploid		No progeny					
Triploid selfed			1	3			4

Since *Petunia* belongs to the Solanaceae, the references to literature are confined chiefly to papers giving accounts of triploidy in plants of this group. Some other papers are referred to for their theoretical application to problems involved.

At the time of writing no publication has been found with any reference to triploidy in *Petunia* and from only two sources (Kostoff, 5; Vilmorin and Simonet, 9) was information obtained concerning tetraploidy. Seeds from large flowering plants were obtained from a dealer (Richard Diener, Oxnard, California) on the possibility that they might be tetraploids. Such proved to be the case when the plants were grown and root tip sections examined. Among these plants, as in mine, both large and small flowered individuals were discovered. These plants were not used in this experiment.

Description and Discussion of Cytological Behavior

For root tips, the paraffin method was used and the slides were stained with crystal violet. Root tips and buds were fixed in Navashin's solution. Some preparations were made from buds by the smear method using either iron-aceto-carmine or fixing in Navashin's and staining with crystal violet. Most of the buds, however, were embedded in paraffin and stained with iron haematoxylin. Root tips were cut 10 microns and buds 18 microns, thick enough to obtain cells with chromosomes intact.

Diploidy

The basic number of chromosomes in *Petunia* is $n = 7$. In somatic cells there are 14 chromosomes (Pl. XXI, fig. 1) that tend to arrange themselves in pairs. They show considerable size difference both in somatic and meiotic figures (fig. 5). Skalinska and Cuchtma (8) studied a number of varieties of *Petunia* and never found plants with more than 14 (diploid) chromosomes. They report some differences in chromosome size in different varieties. Malinowski (7) also reports chromosome size differences in a strain of variegated *Petunia* and finds "on an average the chromosomes of large purple flowers are larger than those of small lilac ones." He suggests the probability of chromosome size being responsible for the occurrence of variegation. The writer has found no significant difference in size of chromosomes between the small flowered and large flowered diploids that were used in connection with this work.

A careful study was made of some phases of diakinesis stages (fig. 2). The pairing of chromosomes was found to be extremely loose, so much so that some pairs showed almost no connection between members. Most of the pairs were found loosely attached at one end, the other ends of the chromosomes being separated from each other. One pair frequently showed both ends attached. Observations of earlier diakinesis showed closer association among some pairs..

Before the nuclear wall is broken in preparation for IM (first metaphase) plate formation the chromosomes thicken and shorten considerably. As soon as the nuclear wall disappears the chromosome pairs seem to be forced to the middle (fig. 3). The pairs do not show any orientation as yet but are rapidly arranged side by side in a group, individuals of the pairs held

end to end, except one pair which often shows two chromosomes attached only at both ends forming an elliptical figure. This last stage is a metaphase shown from side view in figure 4.

Tetraploidy

In a report of chromosome numbers in Solanaceae, de Vilmorin and Simonet (9) briefly mention that *P. violacea* hyb. Hort. has $n = 7$ chromosomes and a variety, superbissima Hort., the multiple 14. This tetraploid variety has very large floral and vegetative parts. On the basis of enlarged parts of the tetraploid plants they conclude that this evidence confirms the opinion of many geneticists that there is a relationship between the size of vegetative and floral organs and the double number of chromosomes. The general rule seems to be that the doubling of chromosome number in a given species is correlated with enlargement of parts of the plant in question. The single tetraploid *Petunia* (14*L*) found among a population of diploids appeared as is expected from doubling of the number of chromosomes, though later when this plant was selfed there were obtained both large and small flowered individuals in approximately a ratio of 3 large to 1 small. Small segregates of this tetraploid plant have flowers and leaves slightly larger and thicker in texture than those of ordinary diploids but not strikingly so. This slight difference between ordinary diploids and small tetraploids may possibly be attributed to doubling of the number of chromosomes. The extreme size of flowers and leaves in large tetraploids, however, certainly cannot be due to the same cause, but rather may be due to a factor for a size character that may have occurred as a point mutation after the chromosomes were doubled, which would explain the 3 : 1 ratio resulting from selfing the original tetraploid (14*L*) plant.

The same situation apparently existed in plants from the seeds obtained from Diener which also segregated into large and small size. Kostoff's plants (referred to below), on the other hand, both the original and its progeny, were small flowered. The situation as found in my own strain of tetraploids (and possibly also in the Diener strain) can be explained by the simultaneous occurrence of doubling of the number of chromosomes and a point mutation for large flowers. Apparently in the case of Kostoff's tetraploid chromosome doubling alone occurred.

Based on these facts the explanation here offered may account for the situation found. However, it is not unlikely that something entirely different from a point mutation may be involved to account for the phenomenon of extreme size of the original 14*L* plant and its large flowered segregates.

Another case of tetraploidy is reported by Kostoff (5) who found a tetraploid plant, among a progeny of diploid *Petunia*, which showed vigorous growth with abundant flowers. Some speculations are made to account for the occurrence of tetraploidy. The author records lagging chromosomes

and delayed reduction divisions leading to diad formation in tetraploids. The tetraploid plant that Kostoff discovered had proved to be completely self-sterile but produced viable seeds when the pollen used was from a diploid. Some of these seeds had been left at the Bussey Institution and they were germinated in the greenhouse. Aceto-carmine smears revealed these plants to be tetraploids instead of triploids. These plants by themselves without contamination from other sources produced abundant seeds but no germination was obtained. No comment is offered in his paper concerning these very interesting phenomena.

In tetraploid somatic cells (fig. 6) chromosomes are found to group themselves in fours, comparable to the condition in diploids where a tendency is shown toward pair formation. Late diakinesis (fig. 7) shows 14 pairs of chromosomes, 12 of which can be made out very clearly. The relationship of a group of four chromosomes is not as clear. All the pairs except the two at the lower left in this figure are attached end to end and the homologous pairs are oriented quite close to each other. This drawing was made from a crystal violet smear and does not show as clearly the connection between the individuals of the pairs as was shown in the drawing of the diakinesis stage of the diploid (fig. 2) which was made from a paraffin section stained with crystal violet. In early diakinesis the same loose pairing was observed as in diakinesis in diploids. Very rarely there were found homologues strung end to end. A case of this kind was observed in a IM plate from side view where a string of four chromosomes were attached end to end.

In the tetraploid it was very difficult to find polar views of the first metaphase plates. The ones found showed chromosomes so compactly arranged together that it was difficult to make counts. Only in one case, from a crystal violet smear, was a good plate found (fig. 8). The difficulty of finding this stage is attributed to loose association of homologous chromosomes resulting in rapid separation of pairs at IM stage.

By close observation, cases of chromosome elimination and also of chromosome splitting were sometimes found, both illustrated in figure 9. Here one plate has 13 large chromosomes and one small; the other plate contains 13 large and 2 small chromosomes and, outside the plate, one small chromosome. It seems in this case as if the two halves of a pair of chromosomes that may have been separated in diakinesis have divided at the first division resulting in four univalents, two of which had gone to one pole, one to the other, and, due to lagging of the fourth, it was eliminated from its destined nucleus. There were observed cases of homologous chromosomes in diakinesis in the three forms studied (diploid, tetraploid, and triploid) being disconnected from each other instead of being in close association. This may be taken as evidence supporting the idea of early division of these single univalent chromosomes. There were observed two cases of non-disjunction where were found two pollen mother cells at IIM

stage that had different numbers of chromosomes at the plates, in one case 16 + 12 and in the other 15 + 13.

Triploidy

The formation of trisomic groups in triploids at diakinesis (fig. 11) and IM was studied and it was found that only rarely did all homologues form 7 trisomic groups (fig. 13). This is unlike the case in *Datura* reported by Belling and Blakeslee (1) where the chromosomes are always shown joining in one way or another in threes. Goodspeed (3) reports bivalents and univalents at IM in triploid *Nicotiana*, and Lesley (6) found trisomes and sometimes univalents at diakinesis in tomato triploids. In *Petunia* at IM where 7 groups of three were observed association in some of these groups was quite loose (fig. 13). More commonly one could count 9 to 11 small and large irregular chromosomes and sometimes as many as 18, rarely 21, if one focused carefully in order to bring out chromosomes hidden behind others. The side view of IM (fig. 12) reveals the peculiar but quite characteristic arrangement of 21 chromosomes. Here is shown one pair eliminated from the metaphase "plate" and the other 19 chromosomes can be made out quite readily. One finds trivalents, bivalents, and univalents, the number of these varying in different cells.

A drawing of diakinesis containing all the chromosomes being difficult, for illustration only a few groups are shown (fig. 11). Grouping here is just as loose as in the diploid. In some the three homologues did not even show any connection but were found not too far distant from each other. Even if we assume there is some connection between these chromosomes, it must be very faint and difficult to make out. Observation on earlier diakinesis showed connection in almost all groups, for the most part end-to-end attachment. In some cases, especially in the group which was seen in the diploid pairing by both ends, the third chromosome was attached only at one end, making various configurations. At later diakinesis commonly one could see a pair with the third chromosome not attached and usually lying close by. The types of association at diakinesis described here would naturally tend to produce a metaphase stage more or less irregular like the one illustrated in figure 12.

It is suggested that the kind of dissociation of homologues observed and discussed above is the cause of irregular metaphase plate formation resulting in irregular divisions to be described.

Observations were made on regular and irregular heterotypic divisions of triploids. Division was considered normal when all three homologues were separated from each other and moved to opposite poles in a random fashion without any lagging, elimination, or dividing of univalents. Division was considered irregular when indication of any of the above peculiarities was observed. Following are the percentages of normal and abnormal divisions in the triploid families:

Triploid 1, 30 percent normal, 70 percent abnormal.
Triploid 2, 58 percent normal, 42 percent abnormal.
Triploid 4, 51 percent normal, 49 percent abnormal.

Counts of chromosome numbers in IIM plates were made to obtain frequency distributions of chromosomes resulting from heterotypic divisions. Results are given in table 2. These counts show a predominance of plates

TABLE 2. *Distribution of Chromosomes in Pollen Mother Cells of Triploids*

No. of Chromosomes	No. of Pollen Mother Cells	% of Pollen Mother Cells
7	1	0.7
8	12	8
9	32	21
10	40	27
11	41	27
12	20	13
13	5	3
14	1	0.7
Total	152	100

with 10 and 11 chromosomes. A typical cell with these numbers is shown in figure 14. Very rarely one could find IIM plates with 7 and 14 (fig. 15). Quite frequently it was found here as in tetraploids that the sum of the chromosomes at the IIM plates was higher than expected, for example 9 + 13, 10 + 12, etc., the sum if more than 21 indicating premature division. Cases showing the reverse, where the sum of the two plates was less than 21, are attributed to elimination of chromosomes.

There were numerous cases where chromosomes, either paired or singly, were eliminated from the plates and found in the plasma (figs. 16 and 19). These cases probably resulted from elimination at the time of formation of the first metaphase plate or possibly from an abnormal heterotypic division, due to a lagging chromosome. Elimination of chromosomes frequently results from irregular behavior at both heterotypic and homotypic divisions (figs. 17, 18, 19, and 20). These lost chromosomes form microcytes which are very numerous in triploid material, occurring quite often in tetraploids and occasionally in diploids. In some cases there have been seen as many as 7 microcytes in a PMC beside the 4 microspores. Very rarely there were seen triads, diads, and occasionally monads either alone or with microcytes. I did not happen to see convincing stages of meiotic division that would explain the origin of the formation of triads, diads, and monads. Some stages were suggestive but a definite conclusion could not be drawn.

Chromosome Counts from Progenies of Back-crossed and Selfed Plants

All possible crosses were made but progeny resulted only from crosses of diploid × triploid, triploid × tetraploid, and from triploids selfed. No

results were obtained from triploid × diploid and tetraploid × triploid. The chromosome numbers of these progeny are given in table 1. From these one may conclude that only those combinations were successful that had chromosome numbers 7 + 7, 7 + 8 in diploid × triploid; 13 + 14 and 14 + 14 in triploid × tetraploid; and 13 + 13 or 12 + 14 and 13 + 14 or 14 + 13 in triploids selfed. Other combinations might have resulted if extreme care had been given in raising seedlings that hardly broke through the seed coat and died readily.

In *Nicotiana*, Goodspeed, Clausen, and Chipman (4) have obtained cross progenies that had intermediate numbers of chromosomes besides plants with parental chromosome numbers. It is interesting to find that both in my case with a diploid-triploid cross and theirs with a triploid-diploid cross the results show that plants with female parental numbers are much more frequent than expected. In *Nicotiana* the back-cross was made one way, triploid ($12_{II} + 12_{I}$) × diploid (12_{II}); out of 34 plants 14 had $36(12_{II} + 12_{I})$ chromosomes like the triploid parent and others had varying numbers. In the experiments reported in the present paper crosses of all possible kinds were made (table 1) but only three ways were found successful. In *Petunia* it may be concluded that the gametes with the chromosome numbers 7, 8, 13, and 14 were more functional than other types, and that a complete haploid set of chromosomes is necessary for gametic development; but in tetraploids the loss of a single chromosome does not prevent the formation of a functional gamete.

In a cross of triploid × tetraploid besides obtaining plants with 27 and 28 chromosomes, there was found one plant with 35 chromosomes which was quite unexpected. In the triploid the somatic number is 21 and in the tetraploid 28. Here appears to be a clear case of non-reduction in triploid from which a megaspore and later an egg with 21 chromosomes appeared which was fertilized by a male gamete with 14 chromosomes, and from their combination resulted a pentaploid plant. This pentaploid plant in its general appearance is more like the female parent, except that there are many flower buds that start to grow but do not develop further, though this peculiarity is not limited to this plant.

Data on Pollen Grains

Measurements

To determine whether any correlation exists between chromosome number and pollen grain size, measurements in microns of 100 to 200 pollen grains from most of the strains available were made. Means of these measurements are as follows: for four diploids, $7S_2 = 36.78 \pm .1053$, $7L_1 = 37.65 \pm .1757$, $7Lnc_2 = 33.02 \pm .1826$, $(7S \times 7L)L_7 = 32.84 \pm .1282$; for three tetraploids, $14S_{2-6} = 39.78 \pm .2107$, $14S_{2-28} = 41.24 \pm .2087$, $14L_{2-25} = 43.62 \pm .1972$; for six triploids, Trip. $S_1 = 36.78 \pm .1843$, Trip. $L_2 = 39.26 \pm .2467$, Trip. $L_3 = 38.38 \pm .2061$, Trip.

$L_4 = 37.94 \pm .1634$, $(14S_{1-2} \times 7L_6)L_1 = 35.75 \pm .1934$, $(14S_{1-2} \times 7L_6)S_2 = 37.87 \pm .3019$.

It will be observed that some relationship exists between number of chromosomes and size of pollen grain, the diploids in general having the smallest pollen grains, the tetraploids the largest, and the triploids intermediate, though the difference between the latter and the diploids is not marked. The classes overlap somewhat and the differences here are not as great as those reported by Belling and Blakeslee (2) and other authors. Strikingly enough, $7Lnc_2$, a somewhat larger flowered plant than $7S$, has comparatively small pollen grains and $(7S \times 7L)L_7$ shows pollen grains smaller than either $7S_2$ or $7L_1$. Apparently flower size and pollen grain size are independent of each other and are no doubt due to different factors. This conclusion is also indicated by the two triploid plants $(14S_{1-2} \times 7L_6)L_1$ and $(14S_{1-2} \times 7L_6)S_2$, the small flowered plant having pollen grains averaging 2 microns larger than the large flowered plant.

In almost all triploids there were found very large pollen grains that may have contained chromosomes in varying numbers from 7 to 14 and higher, and, occasionally, as many as 21. Measurements from the triploid plant $(14S_{1-2} \times 7L_6)S_2$, mentioned above, indicated the presence of some extremely large pollen grains and it was found that 2 percent of the pollen grains measured 60.5–62.7 microns and 1 percent 73.7 microns. It is very probable that these pollen grains contained 21 chromosomes. Tetraploids, due to abnormal division, may also produce small as well as abnormally large pollen grains. A cytological observation of one tetraploid $(14S_{2-6})$ showed a possibility of this kind but in my material no cases were found of tetraploid pollen grains as large as those of the triploid described above.

Counts of Normal and Defective Pollen Grains

Counts of from 100 to 200 normal and defective pollen grains from various plants were made. Newly opened anthers were shaken onto slides and the pollen grains stained in aceto-carmine and stirred before the cover glass was applied. Pollen grains that took a sharp and uniform stain were considered normal and viable. The number and percentage of normal and defective pollen grains were calculated, the latter being divided into two groups, large and minute. The percentages of normal and large defective are calculated from the sum of these two classes, those of the minute defective pollen grains are based on the sum of all three classes. The minute pollen grains being doubtless microcytes, a class by themselves, they were thus calculated separately.

The counts of diploid large defective grains gave percentages from 2 to 14. In the pollen grains from $7S_2$ there were rarely found microcytes and in $7L_1$, 3 percent. In the tetraploids the large defective grains ranged from 6 to 19 percent and microcytes from 1 to 20 percent. In the four original triploids the large defective grains ranged from 19 to 37 percent and microcytes from 11 to 28 percent.

There was observed a close correlation between percentages of abnormal divisions of three triploids (data from Trip. 3 was not available) and the percentages of defective pollen grains of the same three plants. This fact and the fact of the presence of microcytes even among pollen grains from diploid plants suggest the conclusion that the defective pollen grains are the result of microspores that lack at least one of the 7 basic chromosomes.

Summary

1. In diploid *Petunia* plants pairing of chromosomes at diakinesis was found to be extremely loose.

2. Large and small flowered plants in approximately a 3 : 1 ratio were obtained from selfing the original tetraploid plant. Slight increase in size and thickness of flowers and leaves of small flowered tetraploids compared with ordinary diploid plants may possibly be attributed to doubling of chromosome number. Extreme increase in size of flowers and leaves may be due to a point mutation affecting a gene for size and not to doubling of chromosome number.

3. Tetraploids showed loose pairing of chromosomes comparable to the condition in diploids. Rarely there were found four homologues attached end to end at diakinesis and sometimes at IM.

4. The difficulty of finding good IM plates in *Petunia* is attributed to loose pairing and rapid separation of chromosomes of pairs at IM stage. Counts of chromosomes could readily be obtained from IIM plates.

5. Elimination and premature splitting of chromosomes could sometimes be seen in tetraploids.

6. Occasional division of univalents at IM is attributed to the fact that homologues are frequently disjoined at diakinesis or earlier. Cases of non-disjunction were also found.

7. In triploids end-to-end attachment between homologues was the rule at early diakinesis but at late diakinesis univalents were frequently found. At IM viewed laterally, trivalents, bivalents, and univalents were found in varying numbers. Only rarely were 7 trisomic groups formed at IM. It is suggested that this irregular metaphase formation and irregular division are the results of the kind of dissociation of homologues found at diakinesis or earlier.

8. In triploids random assortment at heterotypic division was the rule resulting usually in 10 and 11 chromosomes, rarely 7 and 14, at the poles. Frequently IIM plates were found where the sum of the chromosomes was more than 21. This is attributed to chromosome division at IM. Cases where the sum was less than 21 were due to chromosome elimination.

9. In triploids very rarely there were seen in pollen mother cells triads, diads, and monads either alone or with microcytes. No meiotic figures were found to explain the formation of these cells.

10. All possible crosses of triploids were made but progenies were ob-

tained only from crosses of diploid × triploid, triploid × tetraploid, and triploids selfed. The results show that only gametes with 7, 8, 13, and 14 chromosomes were successful in forming zygotes that attained maturity.

11. A pentaploid plant with 35 chromosomes was obtained from a cross of triploid × tetraploid. This is attributed to non-reduction in the triploid resulting in a megaspore and later an egg with 21 chromosomes which was fertilized by a male gamete with 14 chromosomes.

12. Study of pollen grain size indicated that it was independent of flower size. Some differences in pollen grain size were found between diploids, tetraploids, and triploids but these were not as striking as those reported in other genera.

13. In one triploid 3 percent of extremely large pollen grains were found, probably containing 21 chromosomes.

14. Counts of normal and defective pollen grains were made from diploids, tetraploids, and triploids. Least abnormality was found in diploids and most in triploids.

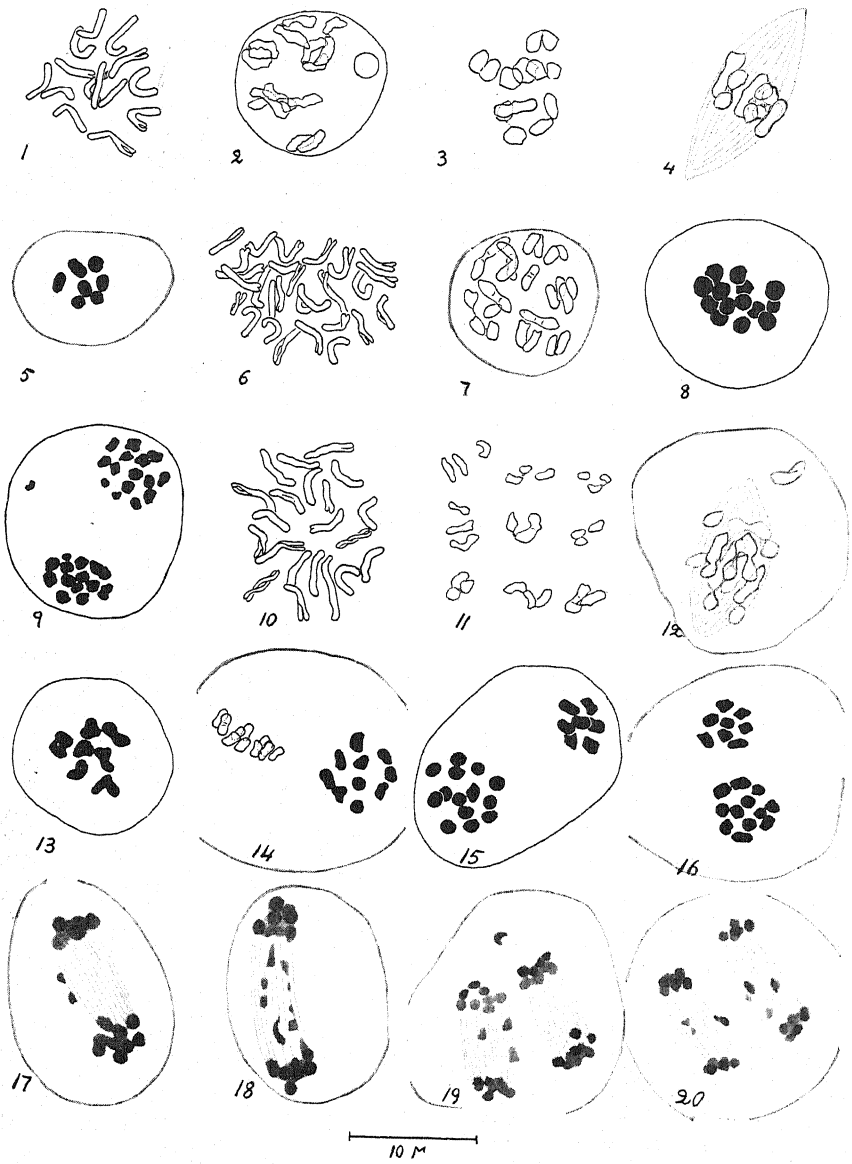
15. An obvious correlation exists between pollen grain abnormality and the abnormal chromosome divisions observed. It is suggested that the cause of defective pollen grains is the lack of one or more of the basic number (7) of chromosomes from microspores.

I am greatly indebted to Dr. E. M. East for placing at my disposal greenhouse facilities, to Dr. Karl Sax for his most helpful criticism and advice through the course of these experiments, and to Mrs. Haig Dermen for her technical and clerical assistance.

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DERMEN: PETUNIA

DESCRIPTION OF PLATE XXI

Diploid, figures 1–5

FIG. 1. $2n = 14$ somatic chromosomes.

FIG. 2. 7 pairs of meiotic chromosomes at diakinesis.

FIG. 3. 7 pairs of meiotic chromosomes after nuclear wall has disappeared and just before a metaphase plate is formed.

FIG. 4. 7 pairs of meiotic chromosomes, homologues attached end to end in a vertical plane at IM stage, side view.

FIG. 5. 7 "bivalent" chromosomes at IM stage, face view.

Tetraploid, figures 6–9

FIG. 6. $2n = 28$ somatic chromosomes.

FIG. 7. 14 pairs of meiotic chromosomes at late diakinesis.

FIG. 8. 14 meiotic chromosomes at IM face view at upper level. There were observed at a lower level 14 chromosomes attached to the upper level but in order to eliminate confusion they were excluded from the drawing.

FIG. 9. IIM stage. One plate contains 13 large and 1 small chromosome, the other contains 13 large and 2 small, and one small chromosome is found in the plasma.

Triploid, figures 10–20

FIG. 10. $3n = 21$ $(7 + 14)$ somatic chromosomes.

FIG. 11. Some typical trisomic groups of chromosomes at diakinesis.

FIG. 12. 21 meiotic chromosomes at IM stage, side view. 19 chromosomes in the middle, 2 in a pair in the plasma.

FIG. 13. 7 trisomic groups at IM stage, face view.

FIG. 14. A typical IIM stage with $10 + 11$ chromosomes.

FIG. 15. A rare IIM stage with $7 + 14$ chromosomes.

FIG. 16. An irregular IIM stage with $8 + 12 + 1$ chromosomes.

FIG. 17. IA stage with 1 pair of chromosomes lagging in the middle.

FIG. 18. IA stage with more than 1 pair of chromosomes lagging.

FIG. 19. IIA stage showing lagging in one and none in the other plate and 1 chromosome in the plasma.

FIG. 20. IIA stage showing lagging of chromosomes at both plates.

THE 50-YEAR PERIOD FOR DR. BEAL'S SEED VIABILITY EXPERIMENT

H. T. DARLINGTON

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The autumn of 1929 marked the 50-year period for the seed viability experiment initiated by Dr. Beal in 1879 at East Lansing, Michigan. Germination tests as recorded in this report were not made, however, until the spring of 1930. A full description of the nature of this experiment has already been published (4). Germination tests were made by Dr. Beal every five years up to 1909, the first report appearing in 1905 (1).

Briefly, the object of the project was to determine the viability of the seeds of some of the common plants (mostly weeds) growing in the vicinity of the Michigan Agricultural College. The first list published contained twenty herbaceous plants and two tree species, namely, *Quercus rubra* and *Thuja occidentalis*. In the tables accompanying the last three tests (including the present) the two trees have been omitted, neither surviving even up to the time of the first test in 1884. Fifty seeds of each of the twenty herbaceous species were mixed in sand, and put in pint bottles. Twenty such bottles were prepared and buried as shown in the figure.

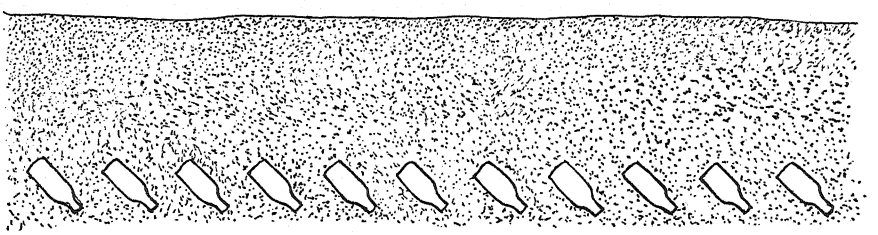
In the first report Dr. Beal shows that eleven of the twenty herbaceous species germinated in the 25th year. Evidently no record was kept of the percentage of germination for each species. The record shows that fourteen different species germinated at some time during the entire period.

The second report (2) shows that eight (possibly nine) species germinated at the end of the thirty-year period. Tests for the next two periods, namely 1914 and 1919, were made by the writer. The test for 1914 was made in the fall. Five (possibly six) species out of the twenty germinated. The original plan was to dig up a bottle every five years. The 40th year came in the fall of 1919, but the test was delayed until the ground had frozen so hard as to make it impossible to dig up a bottle. It was therefore made in the spring of 1920, and the rather unusual results suggested that the wintering over may have been rather fortunate, as the showing was better than in 1914. In his report for 1905, Dr. Beal calls attention to the fact that in the 1894 test he secured germinations in soil kept over an entire year. In 1920, Dr. E. A. Bessey suggested that the interval of testing be lengthened to ten years, thus increasing by sixty years the duration of the experiment.

On April 10, 1930, another bottle was dug up, so that results are now at hand for fifty years. This leaves eleven sets still buried, or enough to last

one hundred ten more years, if the ten-year interval be continued. The buried bottles are about four inches apart in the row, arranged as shown in text figure 1. Careful digging was necessary to find the end bottle and not injure others. A location sheet, giving more detailed directions for exact location was made this last time. One copy of this has been deposited with the Botanical Department of the College, and another with the College Secretary's Office.

The method of testing was essentially the same as described for the 1920 report. The sand was slightly moist when the bottle was taken out



TEXT FIG. 1. Method of burying the seeds in Dr. Beal's experiment.

of the ground. A piece of stiff wire and small amount of water were used to clean the soil from the bottle. Seeds were planted in sterilized soil. In the check flat, each species was kept in a small area of its own. On especially sunny days cheese cloth was spread over the flats to conserve the moisture.

The species planted in the check flat were as follows: *Amaranthus retroflexus*, *Ambrosia elatior*, *Rumex crispus*, *Lepidium virginicum*, *Oenothera biennis*, *Plantago major*, *Chaetochloa lutescens*, *Malva rotundifolia*, *Bursa Bursa-pastoris*, *Alsine media*, *Trifolium repens*, *Agrostemma Githago*, *Bromus secalinus*, *Erechtites hieracifolia*, *Chenopodium album*, and *Anthemis Cotula*. Practically all of these came up and made a thrifty growth.

By April 16, two sprouts of Dr. Beal's seeds became visible, and by April 19, thirty seedlings were counted. Eight days later (April 27), sixty-four seedlings were counted but not identified. The tallest had reached a height of about 2 cm., while the youngest were just breaking through. The seedlings in the check flat appeared about four days sooner and grew more rapidly, so that by April 21, the tallest of the check seedlings was about 5 cm. as against 1 cm. for the others.

The first of the seeds to germinate was *Brassica nigra*, this also being the first to appear in the check flat. By May 1, there were four seedlings of this species. By May 6, twenty-four seedlings of *Rumex crispus* were identified beyond question, and carefully removed to make room for others. On May 14, seventeen seedlings of *Oenothera biennis* were identified and removed. At the end of about ten days some fungous growth appeared on the top of the soil, making it desirable to cultivate the top surface

slightly. This was done May 15, and resulted in the appearance of a number of new seedlings. By May 6, three seedlings had died before they became large enough to be identified. A similar fate overtook two or three others by May 22, and one more June 11.

By May 29, there were twenty seedlings of what were suspected to be *Verbascum Blattaria*, though the species mentioned in the original record was *Verbascum Thapsus*. By June 6, this number had increased to thirty-one, and the seedlings had been definitely identified as *V. Blattaria*. Previous tests, however, have shown that *V. Thapsus* was put in some of the bottles, and no doubt meant to be put in all. If we assume that fifty seeds of this species were originally present, the percentage of germination is 62, the highest of any species for the fifty-year test.

At the conclusion of this test, the total number of seedlings counted was eighty-six, six of which were not identified. About ten of these were produced by cultivating the soil after germination had apparently ceased. In this connection, I wish to quote Dr. Beal's 1905 report. He says, "I have never felt certain that I had induced all the sound seeds to germinate. I moisten the sand containing the seeds and forthwith a goodly number germinate, and then they come slowly straggling along. I dry the soil and after moistening, in a few days more seeds germinate."

On the basis of a thousand seeds to a bottle, the total germination for the 50-year test was evidently 8.8%. A comparison with the 1920 test shows some marked differences in the behavior of the seeds. In 1920, ten species germinated, while in 1930 there were only five. Three of the species, namely *Rumex crispus*, *Oenothera biennis*, and *Brassica nigra* were the same, while *Polygonum Hydropiper* and *Verbascum Blattaria* were not recorded. No explanation is offered for the unusual appearance of the latter. The absence of *Lepidium virginicum* for the first time in the history of the experiment is noteworthy; also the non-appearance of *Amaranthus* which made the best showing in 1920.

TABLE 1. *1930 Test*

Name of Plant	Number of Individuals	Percentage of Germination
Rumex crispus	26	52
Oenothera biennis	19	38
Verbascum Blattaria	31	62
Brassica nigra	4	8
Polygonum Hydropiper	2	4

Table 1 summarizes the results of the test for the 50-year period, giving the number of individuals for each species and the percentage of germination.

Table 2 is a record of those seeds which germinated at one time or other throughout the fifty-year period, the zero sign indicating a failure. Evidently the table does not show the percentage of germination for any

TABLE 2. *Results of All Tests to Date*

Name of Species Tested	5th Yr. 1884	10th Yr. 1889	15th Yr. 1894	20th Yr. 1899	25th Yr. 1904	30th Yr. 1909	35th Yr. 1914	40th Yr. 1920	50th Yr. 1930
Amaranthus retroflexus	+	+	+	+	+	+	o	+	o
Ambrosia elatior	o	o	o	o	o	o	o	+	o
Brassica nigra	o	+	+	+	+	+	+	+	+
Bromus secalinus	o	o	o	o	o	o	o	o	o
Bursa Bursa-pastoris	+	o	+	+	+	+	+	o	o
Erechtites hieracifolia	o	o	o	o	o	o	o	o	o
Chamaesyce maculata	o	o	o	o	o	o	o	o	o
Lepidium virginicum	+	+	+	+	+	+	+	+	o
Agrostemma Githago	o	o	o	o	o	o	o	o	o
Anthemis Cotula	+	+	+	o	+	o	?	o	o
Malva rotundifolia	+	o	o	+	o	o	o	o	o
Oenothera biennis	+	+	+	+	+	+	o	+	+
Plantago major	o	o	+	o	o	o	o	+	o
Polygonum Hydropiper	o	+	+	+	+	?	o	o	+
Portulaca oleracea	o	+	+	+	+	o	o	+	o
Rumex crispus	+	?	+	+	+	+	+	+	+
Chaetochloa lutescens	+	+	+	o	+	+	o	o	o
Alsine media	+	+	+	+	+	+	o	o	o
Trifolium repens	o	o	o	o	o	o	o	o	o
Verbascum Thapsus	+	?	+	+	o	o	+	o	o
(*Verbascum Blattaria*)									+

species, but only the number of times any particular species has germinated in nine tests. The percentage of germination for the forty-year test (1920) was 8.2%, but in this case sprouting was not forced through cultivation.

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STIMULATION PHENOMENA IN THE GROWTH OF BACTERIA AS DETERMINED BY NEPHELOMETRY

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According to the Arndt-Schulz (1) law very weak irritants activate the cell, weak irritants hinder cell activity, strong irritants hinder cell activity, and very strong irritants paralyze the cell. The general truth of this law seems to have been fairly well demonstrated. Raulin (17), who found that the presence of certain metallic salts induced the production of a more abundant mycelium of *Aspergillus niger*, assumed that the salts were necessary for the nutrition of the fungus. Pfeffer (16) later demonstrated that a simple nutrient solution was adequate for the normal growth of the Plectascineae. Richards (18) added minute quantities of various metallic salts to a modified Pfeffer's solution and induced in fungi belonging to this same group the production of a heavier felt than was obtained in the control cultures. Schulz (21) had noted an increased respiration in yeast cultures which he felt was due to the presence of certain poisons in very dilute quantities. An extensive summary of later investigations and excellent bibliographies appear in Czapek (6), Falk (7), and Brenchley (3).

The results of a series of investigations to determine the effect of dilute solutions of the salts of certain heavy metals on the growth of bacteria are presented in the following pages.

METHODS

Organisms

The organisms studied were *Escherichia coli* and *Serratia marcescens*, obtained from the American Museum of Natural History, New York City; *Azotobacter chroococcum*, obtained from Dr. E. B. Fred of the University of Wisconsin, and *Pseudomonas tumefaciens* obtained from Dr. M. Levine of the Montefiore Hospital, New York City.

The stock cultures of *Es. coli* and *S. marcescens* were grown on Bacto-agar, the stock cultures of *A. chroococcum* on Ashby's mannite agar, and the stock cultures of *Ps. tumefaciens* on string bean agar.

Metallic Test Salts

The effects of the following metallic salts were tested: cadmium nitrate, cobalt nitrate, copper sulfate, nickel nitrate, and zinc sulfate. In each case one-tenth volume normal solutions were made and added to the media as required. These salts will be referred to hereafter as the metallic test salts.

Media

The four media employed were chosen because they not only furnished adequate nourishment for the growth of the organisms but also facilitated growth measurements. Merck's reagent chemicals were used throughout, except where otherwise indicated.

Medium *A*. Meat Infusion Agar. Meat infusion agar was used for cultures of *Es. coli* and *S. marcescens*. The basic medium was made according to the following formula:

Bacto-peptone	10 grams
Bacto-beef (dehydrated)	50 grams
Bacto-agar	15 grams
Sodium chlorid	5 grams

To these substances were added either a liter of water, or of the desired zinc sulfate solution. The mixture was autoclaved for fifteen minutes at ten pounds steam pressure. After cooling it was adjusted to plus one, Fuller's scale, cleared with egg albumen and filtered. The solution was placed in media tubes in 10 cc. quantities and sterilized by autoclaving at fifteen pounds pressure for ten minutes.

Media were made up containing the following concentrations of zinc sulfate in terms of normal solutions: .0001; .00025; .0005; .00075; .001; .002; .003; .004; .005.

For each series of experiments, the media for the controls and for the zinc sulfate dilutions were made simultaneously, usually in quantities of 250 or 500 cc.

A medium similar to the above was made up with thirty grams of agar to the liter instead of fifteen. The following concentrations of zinc sulfate were used: .005 *N*, .01 *N*, .025 *N* and .05 *N*. This more concentrated agar was used for *S. marcescens* only.

Medium *B*. A 0.1 percent dextrose broth with peptone, beef, and sodium chlorid in the proportions given for Medium *A* was made for *Es. coli* cultures. The three last named ingredients were dissolved in 900 cc. of water. Active *Es. coli* were introduced, and the mixture incubated at 37.5° C. to remove all fermentable sugars. After twenty-four hours the culture was autoclaved, filtered, and adjusted to plus one Fuller's scale. One gram of Bacto-dextrose was then added. The amount of zinc sulfate required for each dilution was added to a measured quantity of the mixture, after which the volume was adjusted with distilled water.

Media were made up containing the following amounts of zinc sulfate in terms of normal solutions: .0001; .00025; .0005; .00075; .001; .0025; .005; .01.

Twenty cc. of media were placed in Smith fermentation tubes, and sterilized by the discontinuous method. An almost imperceptible colloidal precipitate formed upon the addition of enough zinc sulfate to bring the

concentration up to .0005 *N*. The amount of precipitate increased with the addition of larger quantities of zinc sulfate. As Hotchkiss (13) points out, this reaction is probably due to the precipitation of proteins, peptones, and amino acids by the heavy metal. The metal itself is precipitated in the process. Therefore, the amount of zinc in solution probably varied somewhat from the concentrations indicated above.

The objections to Media *A* and *B* were that there was no accurate hydrogen-ion determination and that many unknown organic compounds in the agar, beef infusion, and peptone may possibly have interfered with the action of the salt on the organisms. The commercial peptones, as is well known, differ one from the other, and it is difficult to obtain any one of them pure in the chemical sense. Unknown impurities, even in minute quantities, may obscure stimulating reaction. For the purposes of the present investigation the writer felt that the use of synthetic media would prove most satisfactory on the assumption that with the nutrient substances reduced to a minimum, and of known chemical composition, the effect of the salts on the organism would be definite enough to admit of measurement. A colorless liquid appeared to be particularly desirable. The two following media were finally selected.

Medium *C*. Ashby's (2) Mannite Solution.

Mannite	15.0 grams
Magnesium sulfate	0.2 gram
Monobasic potassium phosphate	0.2 "
Sodium chlorid	0.2 "
Calcium sulfate	0.1 "
Calcium carbonate	5.0 grams
Water to	1000 cc.

The mannite was Pfansteihl's CP (special d mannitol), and the monobasic potassium phosphate was Merck's (Sörensen's).

When a small amount of the metallic test salt, not more than 5 cc. of tenth normal to the liter, was added to any set in a series, the buffer present was sufficient to prevent any change in the hydrogen-ion concentration, and the amount of solution added was considered to be too small to affect materially the total volume. In such cases the media were made up as follows: The mannite, magnesium sulfate, potassium phosphate, sodium chlorid, and calcium sulfate were weighed out in bulk, and heated in distilled water in an Erlenmeyer flask until completely dissolved. After cooling, the volume was made up to the entire amount needed for the series by the addition of more distilled water, and the hydrogen-ion concentration adjusted to pH 7.5 with normal sodium hydroxid. All hydrogen-ion determinations were made according to the method of Gillespie (10, 11). After thorough shaking, the amounts desired for each dilution of the test salt, and for the control, were measured volumetrically and placed in

separate glass containers. The required amounts of the one-tenth normal solutions of the test salts were then added with a graduated 1 cc. pipette, so standardized that twenty drops equalled 1 cc. at room temperature. The volumes in all the flasks were made equal by the addition of distilled water. The media were placed in 25 cc. quantities in dry sterilized Pyrex Erlenmeyer flasks, each of which contained 0.125 grams of calcium carbonate.

When the test salt was added in greater amounts than 5 cc. to any set of a series, it was necessary to make up and titrate each dilution separately. In such cases the ingredients were added individually, usually in solution form, to each flask of the series, and then the metallic salts, after which the solutions were made up to the required amount in volumetric flasks with distilled water. After thorough shaking, each solution was titrated, and the pH adjusted as indicated above.

Media were made up containing the following salts in the amounts indicated, in terms of normal solutions:

$ZnSO_4$	$CuSO_4$	$MnSO_4$	$CdNO_3$	$CoNO_3$	$NiNO_3$
.000005	.00001	as	as	as	as
.000007	.00005	with	with	with	with
.0000075	.0001	$CuSO_4$	$CuSO_4$	$CuSO_4$	$CuSO_4$
.000008	.0005				
.000009	.001				
.00001	.005				
.00002	.01				
.00003					
.00004					
.00005					
.000075					
.0001					
.0005					
.001					
.002					
.005					
.01					

Medium *D*. The following medium was devised for *Ps. tumefaciens* (5):

Monobasic potassium phosphate (Merck's Sörensen's)	0.5 gram
Magnesium sulfate	0.2 "
Dextrose (bacto)	1.0 "
Asparagine	1.0 "
Water to	1000 cc.

The same procedure was followed as in the case of the mannite solution, except that the pH was adjusted to 6.5 instead of 7.5 in order to avoid a colloidal precipitate which formed in the less acid media.

The following metallic salts were added in the amounts indicated in terms of normal solutions:

$ZnSO_4$	$MnSO_4$	$CuSO_4$	$CoNO_3$ as with $CuSO_4$
.00005	.00005	.000005	
.000075	.0001	.00001	
.0001	.0005	.000025	
.0002	.001	.00005	
.00025	.005	.000075	
.0003	.01	.0001	
.0004		.00025	
.0005		.0005	
.00075			
.001			
.0025			
.01			
.05			

Cultures were grown in media tubes, containing 10 cc. of the solution; in 50 cc. Erlenmeyer flasks, containing 10 cc. of the solution; and in 250 cc. Erlenmeyer flasks, containing 25 cc. of the solution. Great care was taken to have all the media tubes used in one set of equal diameter (5). For the purpose of contrasting growth stimulation under different environmental conditions, media were also made up with five and ten grams of dextrose per liter.

Methods of Measuring Stimulation

Three separate methods were employed for measuring growth; (1) measurement of the amount of gas formed in Smith fermentation tubes, (2) measurement of the size of colonies, and (3) measurement with the nephelometer of the growth indicated by turbidity.

The first method does not measure growth directly, but merely the activity of the cells. It was used with *Es. coli* grown in Smith fermentation tubes containing Medium *B*.

The second method was employed for *Es. coli* and *S. marcescens*. Dilutions of the organisms were made into melted agar, a sufficient number of plates were poured, and the diameters of selected colonies were measured with an ocular micrometer at regular intervals from the time of their first appearance until growth ceased. Special care was taken to inoculate the plates as evenly as possible in order to minimize the errors which might occur as a result of unequal seedings. The number of surface colonies was always counted and considered in the results. The diameter values obtained with the micrometer were converted into millimeters, and the areas of the colonies calculated. The average areas of the controls at any one examination were taken as 100, against which the percent increase or decrease of the colonies in the zinc sulfate cultures were compared.

In the third method the Kober nephelometer made by the Klett Manu-

facturing Company was used to measure turbidity. This method was employed for *A. chroococcum* grown in Medium *C*, and for *Ps. tumefaciens* grown in Medium *D*. As the dead as well as the living organisms contributed to the turbidity, the results were frequently checked by diluting and plating (4).

Experimental Work

Gas Formation in Cultures of Escherichia coli

Smith fermentation tubes containing 0.1 percent dextrose broth were inoculated with varying numbers of *Es. coli* organisms, and incubated at 37.5° C. Observations were made at definite intervals to note the time of and the amount of gas formation in the closed arm of the tube. Comparisons between the controls and the experimental cultures were based upon the time of gas formation and the maximum amount of gas formed at any time in the life of the culture.

Growth and gas formation occurred in the broths (Medium *B*) containing zinc sulfate in the following concentrations: .0001 *N*, .00025 *N*, .0005 *N*, .00075 *N*, and .001 *N*. In the control cultures, and in the cultures containing .0001 *N* and .00025 *N* zinc sulfate, where there had been a very light inoculation, gas first appeared after twenty-four hours; where there had been a heavy inoculation, it appeared in less than twelve hours. In cultures containing .0005 *N* zinc sulfate there was growth after a light inoculation, although it was somewhat delayed in its appearance. With .00075 *N* zinc sulfate there was no growth nor gas formation after a light inoculation, but with a heavier seeding gas ultimately appeared. With .001 *N* zinc sulfate gas appeared later yet, and only when a very heavy inoculation was used.

In the presence of .0001 *N* zinc sulfate a light inoculum produced gas formation similar in amount to that in the controls. With a heavier inoculum more gas was formed than in the control cultures. In the presence of .0005 *N* zinc sulfate less gas was evolved than in the control cultures when a light inoculum was used, but with a heavier inoculum, the amount was about the same as that in the controls. With .00075 *N* and .001 *N* zinc sulfate, less gas was produced regardless of the number of organisms used for inoculations.

Colony Size of Escherichia coli and Serratia marcescens

Es. coli proved to be a more satisfactory organism to work with than *S. marcescens* because its colonies always remained circular and ceased to enlarge after thirty-six hours. *S. marcescens* colonies, on the other hand, were sometimes stellate, and increased in size up to sixty-four hours.

The media for the *Es. coli* series were made as previously indicated. Each tube was melted and inoculated with two or three loops from a twenty-four hour slant growth that had been emulsified and further diluted with

sterile saline. The inoculated tubes were rotated to insure distribution of the organisms, and the agar poured into ordinary petri dishes of uniform size and shape. In each series of experiments two plates were prepared for the controls and for each zinc sulfate concentration. When the agar hardened the plates were inverted and incubated at 37.5° C. for eight hours. Selection of colonies for study was simplified by calculating the dilutions so that growth after eight hours yielded about ten isolated surface colonies, approximately equidistant, just visible to the naked eye.

With the aid of the microscope, the top of the dish being removed when necessary, ten selected colonies were marked with numbers on the under side of the dish. The diameters of these colonies were measured with an ocular micrometer. The selected colonies were measured again after twelve, sixteen, twenty, twenty-four, and thirty-two hours of incubation, by which time they had ceased to increase in diameter. The results are shown in table 1. Each figure represents the average obtained from seven

TABLE 1. *Size of Escherichia coli Colonies Grown on $ZnSO_4$ Agar*

Conc. of Salt	Number of Hours Incubation												Average Number of Surface Colonies
	8		12		16		20		24		32		
	A *	B †	A	B	A	B	A	B	A	B	A	B	
Control.....	.42	100	1.17	100	2.32	100	3.63	100	4.53	100	5.81	100	180
.0001 *N*.....	.37	89	1.17	100	2.34	101	3.85	106	4.78	106	6.33	109	178
.00025 *N*.....	.42	99	1.26	108	2.51	108	3.99	110	4.96	109	6.33	109	177
.0005 *N*.....	.46	111	1.34	115	2.70	116	4.22	116	5.33	118	6.83	118	181
.00075 *N*.....	.50	118	1.23	105	2.36	102	3.62	100	4.29	95	5.58	96	178
.001 *N*.....	.55	131	1.31	112	2.47	107	3.79	104	4.30	95	5.58	96	182
.002 *N*.....	.62	147	1.27	109	2.33	100	3.42	94	4.18	92	5.27	91	185
.003 *N*.....	.67	158	1.40	120	2.53	109	3.77	104	4.52	100	5.87	101	175
.004 *N*.....	.65	155	1.33	114	2.53	109	3.83	106	4.64	102	6.21	107	173
.005 *N*.....	.60	143	1.15	98	2.35	101	3.34	96	4.07	90	5.02	96	186

* *A* = area in square millimeters.

† *B* = area in terms of control.

Each figure in columns *A* and *B* is the average obtained from 140 individual colonies.

separate series, each one of which was examined in the manner just described, that is, each figure represents the average of one hundred and forty individual colonies.

The average areas for the controls and the zinc sulfate cultures are shown in the columns headed *A*. The data in the *B* columns were obtained by considering the area measurements of the controls of each examination as 100 and calculating the ratios of the other areas to the controls. The normal growth curve for the control cultures and those containing the salts may be plotted from the figures for each hour under *A*, reading from left to right. The average number of surface colonies counted after thirty-two hours incubation was apparently sufficiently constant to minimize any possible effect of difference in number.

On the basis of the figures one may conclude that with the zinc sulfate

concentrations of .001 *N*, .002 *N*, .003 *N*, .004 *N*, and .005 *N*, there was a distinct stimulation in the growth of the colonies, followed by an apparent inhibition. Where the greatest initial stimulation occurred, the inhibition was not sufficient to overcome the initial stimulation, and the colonies never returned to "normal." The greatest stimulation produced was 58 percent in the .003 *N* agar after eight hours incubation. With weaker concentrations of zinc sulfate, .0001 N and .00025 *N*, there appeared to be an initial inhibition followed by stimulation which resulted in the formation ultimately of larger colonies than the controls. The greatest stimulation was 10 percent with the .00025 *N* agar after twenty hours incubation. In the cultures with zinc sulfate concentrations between .00025 *N* and .001 *N* the salt appeared to produce only slight initial stimulation and very little toxic effect. Cultures with agar containing still more zinc sulfate than .005 *N* showed no stimulation and an increasing toxic effect.

The evidence seems to indicate what might be considered a double zone of growth stimulation. The phenomenon of initial stimulation due to toxic agents in dilute quantities, followed by a return to normal, or by inhibition, is one commonly observed. The occurrence in the presence of still more dilute solutions of the poison of a zone of initial inhibition, followed by stimulation, has apparently not been noted. The phenomenon may have been overlooked because observations were made with insufficient frequency, or because it exists only under the special environmental conditions provided.

The results obtained by growing *S. marcescens* in a similar manner to that described for *Es. coli*, and with the same zinc sulfate dilutions, were not so clear cut. The method was varied slightly because of the difference in the two organisms. The plates were incubated at 25° C., and examined after sixteen, twenty, twenty-four, twenty-eight, thirty-two, forty, and forty-eight hours. Table 2 shows the results of four sets, each made up of

TABLE 2. *Size of Serratia marcescens Colonies Grown on $ZnSO_4$ Agar*

Conc. of Salt	Number of Hours Incubation														Average Number of Surface Colonies
	16		20		24		28		32		40		48		
	A *	*B* †	*A*	*B*	*A*	*B*	*A*	*B*	*A*	*B*	*A*	*B*	*A*	*B*	
Control..	.09	100	.63	100	1.83	100	3.80	100	5.47	100	7.48	100	8.34	100	149
.0001 *N*..	.09	102	.59	94	1.77	97	3.76	99	5.35	98	7.55	101	7.99	96	140
.00025 *N*.	.08	82	.79	125	1.86	102	4.52	119	6.03	110	7.94	106	8.34	100	127
.0005 *N*..	.12	112	.66	105	1.98	108	4.23	111	5.56	102	7.35	98	7.74	93	129
.00075 *N*.	.06	68	.45	71	1.63	89	3.63	96	4.91	90	6.88	92	7.21	86	146
.001 *N*...	.09	95	.57	90	1.98	108	4.41	116	5.98	109	8.81	118	9.18	110	142
.002 *N*...	.10	105	.58	92	1.77	97	3.84	101	5.52	101	7.95	106	8.35	100	130
.003 *N*...	.15	132	.74	118	2.56	140	4.87	128	6.34	116	8.76	117	9.02	108	146
.004 *N*...	.19	172	.92	146	2.08	153	5.60	147	7.02	128	8.40	112	8.81	106	119
.005 *N*...	.11	110	.58	92	1.83	100	3.56	94	4.75	87	7.50	100	8.35	100	128

* *A* = area in square millimeters. † *B* = area in terms of control.
Each figure in columns *A* and *B* is the average obtained from 80 individual colonies.

two plates of ten colonies for each dilution. Every figure is the average of measurements on eighty colonies. There was a greater irregularity in the number of surface colonies than with *Es. coli*, but this variation apparently had no appreciable effect on the results.

Another set of five series was run through, where each measurement was the average of one hundred individual colonies. It agreed in general with the results given in table 2.

It is obvious, despite the irregularities, that for *S. marcescens* there is a zone in the zinc sulfate concentrations in which an initial growth stimulation was produced, followed by a return to conditions approaching normal. The optimum concentration of zinc sulfate for growth stimulation was .004 *N*. This concentration produced a growth increase of 72 percent after sixteen hours incubation.

There is little evidence here of the effect noted with *Es. coli* where very weak zinc sulfate dilutions caused an initial growth inhibition, followed by a growth stimulation.

The apparent increase in size near the end of the growth period in *S. marcescens* is probably due to the formation in some cases of stellate colonies which rendered exact measurements difficult. The size was evidently over- rather than under-estimated. There seemed to be no direct correlation between the irregular shaped colonies and the different zinc sulfate solutions, although the colonies of the control cultures remained circular.

In another set which was run through in triplicate on 3 percent agar instead of the usual 1.5 percent, the effect of the stiffer medium was pronounced. In the control plates the colonies developed later and more slowly and did not attain the ultimate size they did in the plates with the less concentrated agar. In the .005 *N* zinc sulfate cultures with the 3 percent agar there was no primary stimulation like that which occurred when the 1.5 percent agar was used. This result was due perhaps to the fact that with the increased agar the sol phase was lessened, and consequently the salts contained therein were more concentrated and therefore more toxic. The .025 *N* zinc sulfate agar showed no growth until twenty hours incubation, and the .05 *N* agar none until forty hours incubation. In each case the colonies produced were very small, 8 percent of the normal size with the .025 *N* and 3 percent of the normal size with the .05 *N* after seventy-two hours growth.

Mention may be made at this point of the phenomenon of pigment formation. In the controls the colonies had the typical brick red color characteristic of *S. marcescens*. When increasing amounts of the zinc sulfate were added to the media, the colonies showed a corresponding tendency to become cerise, so that it was possible to distinguish the different plates by the varied pigmentation alone.

Growth Measurement with the Nephelometer

Azotobacter chroococcum

The growth of *Azotobacter* on synthetic media is never as luxuriant as it is when small quantities of humus are present. When the organism is grown in Erlenmeyer flasks containing Ashby's mannite solution, there is at first a slight general cloudiness, which later increases, and a translucent zoögleal mass forms at the junction of the solution surface and the container wall. Still later a fine white sediment forms which turns light brown and ultimately becomes dark brown. In no case is there formed a true surface scum or pellicle.

In the investigation here reported the actual growth in a synthetic medium to which dilute solutions of metallic test salts had been added was measured nephelometrically to determine the presence or absence of stimulation.

For inoculation, the organisms from a forty-eight hour mannite agar slant were transferred to a 500 cc. flask containing the mannite solution medium. This inoculated medium was then well shaken and incubated at room temperature (22–23° C.) for twenty-four hours, at the end of which time it was a cloudy white due to the growth of the organisms. Ten drops of this culture were placed with a sterilized standard 1 cc. pipette into each 250 cc. flask containing the experimental media. An inoculum containing fewer organisms was unsatisfactory. The inoculated flasks were well shaken, and incubated at room temperature. Observations on the first appearance of turbidity, and later, on the relative degrees of turbidity, were made with the naked eye and checked by nephelometric readings.

The actual life of a single culture of this sort is about twenty-five days. Individual cultures were not destroyed at the time when they were examined. Instead, small portions of each culture were removed at definite intervals, according to the following procedure. The flasks were thoroughly shaken fifty to seventy-five times, and where the organisms had attached themselves to the sides of the container they were freed with a platinum loop. One cc. was removed with a sterile 1 cc. milk pipette and placed in a test tube containing 4 cc. of distilled water. This mixture was turbid, due not only to the organisms in it, but also to the presence of the calcium carbonate. The carbonate completely disappeared on the addition of five drops of normal hydrochloric acid, and the acid, rather than tending to agglutinate these organisms, seemed to aid in their dispersal. The material so treated was then ready to be tested nephelometrically.

With the younger cultures, up to ten days old, the nephelometer was found to be satisfactory. However, in older cultures, as the zoögleal condition of the cells became more marked, the clumps were more difficult to break up, and the results were correspondingly less accurate. The formation of pigment during the later stages of growth also tended to complicate

the readings and their interpretations. Colony counts to check up the nephelometric findings were not made because the zoögleal character of the organisms would have rendered the results inaccurate.

The procedure for the nephelometric examinations in all cases was as follows: The series was divided into as many sets as there were flasks of each concentration. For example, if the set was made in triplicate, the flasks were divided into three complete single sets. Each of the sets was examined as a unit. The control culture in a given series was taken as the standard, set at fifteen in the nephelometric scale, and each of the other cultures in the series was in turn compared with it. The figures for the three sets so obtained were then averaged. The results, while relative, served to show adequately the presence and amount of growth in the experimental and control cultures.

The controls were always tested one against the other at the time when the nephelometric readings were taken, and found to vary never more than 15 percent at any one time.

Four series of flasks were set up to determine the effect of zinc sulfate on growth. In each series there were two or three, and in one, seven flasks of each dilution. In brief, the results based on the appearance of turbidity after the carbonate had been dissolved, showed that growth stimulation occurred in the presence of .000005 *N* and .000007 *N* zinc sulfate; that with stronger concentrations, growth was initially inhibited but later stimulated; and that no growth whatsoever occured with .005 *N* zinc sulfate. The maximum stimulation, 22 percent, occurred with .000009 *N* zinc sulfate after five days incubation.

In series one and two zinc sulfate stimulation was not apparent. Series three showed definite evidence of stimulation. The averaged results are presented in table 3. They indicate that growth was constantly stimulated

TABLE 3. *Growth of Azotobacter chroococcum in Ashby's Solution with Dilute Solutions of $ZnSO_4$. Series III*

Number of Days Incubation

Conc. of Salt	1	3	5	7	10	15
Control	100	100	100	100	100	100
.000007 *N*	109.4	103.3	117.0	113.4	108.8	116.1
.000008 *N*	78.0	92.0	101.3	87.4	103.1	101.5
.000009 *N*	60.0	77.2	122.4	96.0	103.4	108.5
.00001 *N*	62.0	100.0	121.0	106.4	98.9	100.1
.00002 *N*	——	68.4	61.5	64.6	79.5	91.9
.00003 *N*	——	——	——	——	55.9	76.5

by .000007 *N* zinc sulfate; that with .000009 *N* and .00001 *N* there was an initial growth inhibition followed by a stimulation; and that with higher concentrations growth was always sub-normal. In series four seven flasks of each dilution were used. The results showed a growth stimulation with .000005 *N* zinc sulfate, and practically none with .0000075 *N*.

Of the metallic test salts used, other than zinc sulfate, manganese sulfate was the only one producing an increased growth in the concentrations used, that is, .00001 *N* to .005 *N* (see table 4). The maximum stimulation, 19

TABLE 4. *Growth of Azotobacter chroococcum, Based on Nephelometric Readings of Relative Turbidity, in Ashby's Solution plus* $MnSO_4$

Number of Days Incubation

Conc. of Salt	2	5	10	15	20	25
Control	100	100	100	100	100	100
.00001 *N*	113.4	102.5	90.8	98.4	83.2	85.0
.00005 *N*	118.6	102.5	110.5	98.4	88.6	——
.0001 *N*	102.6	102.3	95.8	93.8	84.4	——
.0005 *N*	105.6	988	94.7	88.7	86.2	78.7
.001 *N*	94.1	101.3	102.4	89.9	86.9	82.4
.005 *N*	——	58.7	64.6	61.1	71.6	59.7
.01 *N*	——	——	——	——	——	——

percent, occurred with .00005 *N* manganese sulfate after two days incubation. The approximate order of decreasing toxicity of all the metallic salts used with *A. chroococcum* as shown by the highest concentrations in which no growth occurred were: Magnesium sulfate .01 *N*, zinc sulfate .005 *N*, cobalt nitrate .0001 *N*, copper sulfate .001 *N*, nickel nitrate .00005 *N*, and cadmium nitrate .00005 *N*.

A microscopic examination of the organisms in the *Azotobacter* cultures was made after fifteen days incubation. The cells were examined both in the living state as seen in the hanging drop and stained with methylene blue. The cells in the control cultures were always evenly distributed. They had typical gelatinous walls and contained indistinct granules. The cells in the zinc sulfate and manganese sulfate cultures which produced normal or stimulated growth were like those in the control cultures. The cells in the test salt cultures where growth was somewhat inhibited were smaller, less rounded and more granular. Where the salts were present in still more toxic concentrations, some of the cells were very large, containing highly refractive granules, some were of normal size, and there were in addition tiny gonidial-like forms (15).

In some of the cultures containing test salts, pigmentation began to be evident on the fifteenth day. It was not present in the controls, however, until the twentieth day. Jones (14) says that the pigment in cultures of *A. chroococcum* on Ashby's mannite solution is produced apparently only when there is a lack of suitable nutrient material, and where the organisms have ceased to multiply. Rippel and Ludwig (19) working with the same organism report that the originally colorless colonies darken as the result of the oxidation of tyrosine to melanin, the tyrosine being formed in the breaking down of proteins when the carbohydrate supply is insufficient or unsuitable. In the present investigations pigmentation was first evident,

and later became most marked in cultures containing concentrations of the test salts which were nearly lethal, not in those producing growth stimulation. It seems unlikely in this case that the carbohydrate supply was exhausted. Instead, it is probable that the presence of the metallic test salt prevented in some way the use of the carbohydrate by the bacteria.

The cultures which darkened soonest and most deeply in the presence of the salt in toxic concentrations seemed to have completed their culture cycle more rapidly than the normal cultures or those known to contain growth stimulating concentrations of the salts. It would appear that the concentrations of the salts producing growth stimulation and life cycle stimulation were not the same. The early completion of the culture cycle is perhaps comparable to the early production of spores by fungi growing in an unfavorable environment, and the stunted growth and early fruiting of higher plants when bad ecological conditions prevail. It would be incorrect to regard such reactions as evidence of growth stimulation. In attempting to arrive at conclusions with regard to the latter one must apparently abide by the criteria of true growth and cell division.

A. chroococcum cells in Ashby's solution showed a sensitivity to the metallic salts greater than that of many organisms in liquid synthetic media. In the case of zinc sulfate, for example, the greatest growth stimulation appeared in the .000009 *N* solution, whereas the favorable concentration for *Ps. tumefaciens* (see p. 280) was .00005 *N*, for *Es. coli* .0001 *N*, for *Sterigmatocystis niger* and *Penicillium glaucum* .000125 *N* to .005 *N* (18) and for the cytoplasmic streaming of *Elodea* and *Chara* .0003 *N* and .001 *N*, respectively (20). In the case of the copper sulfate the dilution producing stimulation is not known. A solution containing .00001 *N* is slightly inhibiting. Fred (8) states that he found *Azotobacter* extraordinarily susceptible to copper in the form of copper sulfate in his cultures. Algal cells are very sensitive, especially to salts like copper sulfate, which has almost a specific toxicity for them. *A. chroococcum* in liquid culture media might be classed with the algae in sensitivity to metallic salts, the bacteria, the fungi, and the yeasts being less sensitive.

Pseudomonas tumefaciens

With the hydrogen-ion concentration initially somewhere between pH 6 and 8, it was found that the growth of *Ps. tumefaciens* was hastened with increasing temperature, up to 28° C., with a more available oxygen supply, and with an increase in the amount of dextrose (5).

It has already been pointed out that a colloidal precipitate formed when metallic salts in certain concentrations were added to the adjusted media. Attempts to eliminate the precipitate by the addition of a few drops of one-tenth normal hydrochloric acid to all the tubes before taking the readings were not wholly successful because an acid agglutination of the organisms occurred, which, in turn, affected the readings. As the precipitate increased

the solids present in the culture tubes, and therefore rendered the nephelometric readings inaccurate, it was necessary to note the amount of the precipitate and where it was formed. A .0001 *N* and .00025 *N* solution of zinc sulfate in the medium produced a very slight colloidal aggregation, insufficient, however, to affect the readings. When a .0005 *N* solution of the same salt was used, a slight precipitate formed when the cultures were left standing, and a .00075 *N* solution produced a considerable precipitate. In both cases the flocculent mass was sufficient to make the readings inaccurate. In the case of manganese sulfate, a .0001 *N* solution brought about a very slight colloidal aggregation, but only with the .0005 *N* and .01 *N* was there a sufficient precipitate to affect the readings. No precipitate formed with the other salts in the dilutions used. When the growth in the cultures was relatively slight, the precipitates noted above were often sufficient to throw the nephelometric readings off. When, however, the growth of the organisms produced a heavy cloud, the precipitate was marked, and the readings much more nearly accurate. These points will be noted more in detail later on.

In making up cultures, organisms from a heavily seeded twenty-four hour growth on bean agar were transferred to a 500 cc. Erlenmeyer flask, containing 50 cc. of media. The flask was well shaken, and then incubated at 28° C. for twenty-four hours. A turbid culture containing approximately 700 million organisms per cc. resulted. Five drops of this culture, removed with a sterile calibrated 1 cc. pipette, were used as the inoculating unit.

The control cultures were used as standards. In some of the preliminary experiments, 1 cc. was removed from large flasks of the cultures and diluted for nephelometric readings. It was found advantageous, however, to set up smaller culture units, and to examine a certain number of them at stated intervals.

After readings were made on a series of tubes to which no acid had been added, the contents of the control tubes and the respective contents of each zinc sulfate group in the series were pooled. The resultant mixtures, which both by microscopic examination and plate counts were shown to contain living, motile organisms, were used for determinations of the sugar content, the hydrogen-ion concentration, and the number of organisms present. Hydrochloric acid was added to only a portion of a given culture whenever it became necessary to dissolve the precipitate mentioned above. The remnants of such cultures were pooled for subsequent examination.

The environmental conditions producing optimum growth stimulation by zinc sulfate were first determined and the work with the other metallic test salts was carried on under these same optimum conditions. The technique was similar to that employed in the experiments with *Azotobacter*.

Zinc Sulfate

Ps. tumefaciens, grown in flasks at 22° C., was stimulated by the presence of .0001 *N* zinc sulfate, was slightly inhibited by .00075 *N*, and almost completely inhibited by .01 *N*.

The hydrogen-ion concentration and the titrible acidity of the zinc sulfate cultures were practically the same as in the controls. The amount of dextrose consumed by the organisms in the more dilute zinc sulfate cultures, .00005 *N*, .000075 *N*, and .0001 *N*, paralleled that in the controls. In the cultures containing greater amounts of zinc sulfate, where the growth of the organisms was retarded, the sugar disappeared later than in the controls. The fact that the sugar was used up at the same time in the control and stimulated cultures might indicate more efficient assimilation of the carbohydrate in the presence of dilute solutions of the salt.

Effect of $ZnSO_4$ on Cultures Incubated at 22° C. and 28° C. Tubes containing 10 cc. of media, grown at 28° C., produced about twice as many organisms as those incubated at 22° C. To determine the effect of zinc sulfate under these two temperature conditions, five large series of tubes were set up containing, with the controls, the following dilutions of the salt: .00005 *N*, .000075 *N*, .0001 *N*, .00025 *N*, .0005 *N*, and .00075 *N*. In each series, identical sets were incubated at 22° C. and 28° C. After five, seven, and ten days, eight tubes of each dilution at each temperature were removed and examined. The results are shown in table 5, where each figure is the average of forty readings.

TABLE 5. *Growth of Pseudomonas tumefaciens in Medium D with Dilute Solutions of $ZnSO_4$. Effect of Incubation at Different Temperatures on the Basis of Relative Turbidity, Measured Nephelometrically*

Conc. of Salt	Number of Days Incubated					
	5		7		10	
	Incubation Temp.		Incubation Temp.		Incubation Temp.	
	22°	28°	22°	28°	22°	28°
Control	100	100	100	100	100	100
.00005 *N*	107.6	117.0	103.7	119.5	100.5	115.2
.000075 *N*	95.7	83.1	93.1	84.6	91.0	92.1
.00001 *N*	99.4	90.9	100.7	86.6	94.4	89.4
.00025 *N*	94.4	91.8	97.4	93.8	94.6	95.0
.0005 *N*	100.0	88.4	97.1	91.1	103.0	97.6
.00075 *N*	111.1	70.0	104.9	79.1	105.4	97.0

Each figure represents the average of the readings on 40 cultures.

The apparent stimulation in the more concentrated zinc sulfate solutions at 22° C. is due to the presence of the colloidal precipitate, which is masked by the greater growth at 28° C. There was, however, at each temperature a distinct zone of true stimulation in the presence of .00005 *N* zinc sulfate,

which was greater and of longer duration in the cultures grown at the higher temperature, i.e., at 28° C. After two, seven, and ten days there was an increase of 17, 20, and 15 percent, respectively, while for 22° C. an increase of 8, 4, and 0.5 percent. Colony counts of these same cultures did not indicate the presence of the stimulating zone very satisfactorily. They did show, however, the zone of relative toxicity.

Effect of $ZnSO_4$ on Cultures Having Different Oxygen Tensions. The effect of zinc sulfate upon *Ps. tumefaciens* under conditions of varying oxygen tension was brought out in three series of experiments. In each experiment 10 cc. of media were placed in media tubes and in 50 cc. Erlenmeyer flasks, and incubated at 28° C. After two and five days, six to ten tubes and five to six flasks for each dilution used were removed and examined. By the third day the media in the flasks began to turn yellow, and by the fifth day were a dirty brown, particularly in the flasks where there had been the greatest growth. The color made it difficult to compare the solutions in the nephelometer without further treatment. However, by diluting 1 cc. from the culture flask with 4 cc. of water, the pigmentation was rendered practically negligible. The turbidity could then be measured with fair accuracy. The same dilutions of the salt were used as with the temperature series. The results, determined nephelometrically, are shown in table 6. There was no growth stimulation with the greater oxygen

TABLE 6. *Growth of Pseudomonas tumefaciens in Medium D with Dilute Solutions of $ZnSO_4$. Effect of Varying Oxygen Tensions on the Basis of Relative Turbidity Measured Nephelometrically.*

Conc. of Salt	Number of Days Incubated			
	2		5	
	A	*B*	*A*	*B*
Control	100	100	100	100
.00005 *N*	121	100	109	102
.000075 *N*	94	94	99	90
.0001 *N*	95	90	97	95
.00025 *N*	125	89	103	97
.0005 *N*	124	86	95	90
.00075 *N*	131			

A = 10 cc. of medium in tubes.
B = 10 cc. of medium in 50 cc. Erlenmeyer flasks.
Each figure under *A* represents the average of the readings on 25 cultures.
Each figure under *B* represents the average of the readings on 15 cultures.

tension, i.e., in the flask cultures. There was a stimulation of 21 percent after two days incubation with a .00005 *N* zinc sulfate in the tube cultures where less oxygen was available. The apparent growth stimulation in the more concentrated zinc sulfate cultures was due to the presence of the precipitate in the media.

Effect of $ZnSO_4$ plus Various Amounts of Dextrose. Two series of cultures were run, each composed of three sets of tubes containing the same zinc sulfate dilutions used in the other experimental work, but varying from each other in the amount of dextrose present, namely, 0.1, 0.5, and 1.0 per cent. All cultures were incubated at 28° C. and six to eight tubes of each salt dilution with each of the sugar concentrations were removed for examination after the two, five, and ten days. Each set was measured against its own control, and the controls one against the other. The results of the nephelometric readings are shown in table 7.

TABLE 7. *Growth of Pseudomonas tumefaciens in Medium D with Dilute Solutions of $ZnSO_4$ Effect of Varying Amounts of Dextrose on the Basis of Relative Turbidity, Measured Nephelometrically*

Number of Days Incubated

Conc. of Salt	2			5			10		
	Percent Dextrose			Percent Dextrose			Percent Dextrose		
	0.1	0.5	1.0	0.1	0.5	1.0	0.1	0.5	1.0
Control	100	100	100	100	100	100	100	100	100
.00005 *N*	98	97	88	106	91	91	102	110	107
.000075 *N*	95	89	81	97	84	75	96	97	94
.0001 *N*	99	89	76	91	80	72	100	102	96
.00025 *N*	106	92	72	96	80	76	99	103	91
.0005 *N*	119	88	76	97	74	72	90	100	97

Each figure represents the average of the readings on 15 cultures.

The apparent inhibition with the .00005 *N* zinc sulfate dilution with 0.1 percent dextrose after two days incubation, is not normal. It occurred in only one of the series, but it was sufficient to affect the average. The stimulation which seems to have existed in the 0.5 and 1.0 percent sugar cultures on the tenth day may or may not be real. The pellicle formation at that time was heavy, and there is a possibility that the indicated results are referable to inaccuracies in the technique.

The definite growth stimulation which normally occurred with 0.1 percent dextrose and .00005 zinc sulfate after two and five days incubation, did not occur when the amount of dextrose was increased to 0.5 and 1.0 percent. There was, on the contrary, a growth inhibition.

All of the above experiments with *Ps. tumefaciens* in cultures with zinc sulfate show that the cultures which offer the optimum conditions for the maximum number of organisms produced during the culture cycle, i.e., partial oxygen tension, incubation at 28° C., with 0.1 percent dextrose, also offer conditions optimum for the stimulation effect of dilute solutions of zinc sulfate. Probably at no time during the culture cycle of the organisms growing under the conditions given above is the logarithmic rate of increase

as rapid as when the oxygen supply is greater or when there is an increased sugar supply: i.e., the most active metabolism and the greatest susceptibility are not coincident. It has already been shown by Frouin (9), who duplicated with variations the work of Raulin (17), that if the carbohydrate nutrition was altered the apparently favorable actions of zinc and iron disappeared. Slight changes in environmental conditions therefore may possibly result in the suppression of growth stimulation.

Manganese Sulfate

The entire set of tubes and flasks of the manganese sulfate series were inoculated at the same time, from an inoculum containing approximately 600 million organisms per cc. by plate count. All cultures were incubated at 28° C. Eight or nine tubes and six flasks were used for each examination. The tubes were tested nephelometrically after two, five, and ten days. It had been noted previously that there was a heavy precipitate in the media containing the more concentrated solutions of this particular test salt. Therefore, when the culture tubes were ready to be examined, 5 cc. quantities were removed from each control and from each dilution of the metallic test salt and pooled in separate receptacles for subsequent examination. To what was left in the tubes five drops of normal hydrochloric acid were added, and the nephelometric examination made as soon as possible, to avoid complications arising from agglutination of the organisms. The pooled material was tested for the hydrogen-ion concentration and the

TABLE 8. *Growth of Pseudomonas tumefaciens in Medium D with Dilute Solutions of $MnSO_4$. Effect of Varying Oxygen Tensions*

Number of Days Incubated

Conc. of Salt	2						5						10		
	A			*B*			*A*			*B*			*A*		
	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3
Control	100	409	100	100	944	100	100	660	100	100	1,056	100	100	732	100
.00005 *N*	101	411	101	86	850	90	99	598	91	101	1,174	111	98	742	100
.0001 *N*	108	403	99	78	686	73	105	557	84	101	1,176	110	104	720	98
.0005 *N*	118	458	112	87	870	92	128	621	95	97	1,197	113	109	555	76
.001 *N*	107	453	111	83	776	82	106	556	84	91	1,141	108	102	459	63
.005 *N*	62	209	51	78	792	84	99	485	74	97	1,124	106	96	504	69
.01 *N*	55	250	60				59	244	37				61	220	30

A = 10 cc. of medium in tubes.
B = 10 cc. of medium in 50 cc. Erlenmeyer flasks.
1 = relative turbidity measured nephelometrically.
2 = million colonies per cc.
3 = relative number of colonies in terms of control.
Each figure under *A* represents the average of the readings on 8 cultures.
Each figure under *B* represents the average of the readings on 6 cultures.

amount of sugar used. Subsequently two plates for each concentration of the salt were made for colony counts. The flasks which were examined after two and five days were treated in the same way, that is, 3 cc. were removed from each flask and pooled, and six drops of hydrochloric acid were added to what was left. The nephelometric examination showed no growth stimulation in the flask cultures. The results in this respect paralleled the findings with zinc sulfate. In the tubes, however, the presence of .0005 *N* manganese sulfate apparently brought a growth increase of 18 percent after two days, 28 percent after five days, and 9 percent after ten days. Colony counts made from the tube cultures verified the nephelometric findings, in that the .0005 *N* concentration of the salt produced the greatest growth increase, 12 percent after two days. The results are shown in table 8. The parallelism between the colony count and the nephelometric readings is striking, and what one would expect from a theoretical consideration of the matter.

Cobalt Nitrate and Copper Sulfate

The cobalt nitrate and the copper sulfate series were inoculated from a culture containing approximately 900 million organisms per cc. by plate count. Nine tubes of each salt concentration and nine controls were removed for examination after two, five, and ten days. The results obtained by nephelometric readings are tabulated in table 9. Both salts in the proper concentrations produced a growth stimulation which reached its maximum after five days incubation. The increase was 14 percent with the .000025 *N* cobalt nitrate, and 52 percent with the .00005 *N* copper sulfate.

TABLE 9. *Growth of Pseudomonas tumefaciens in Medium D with Dilute Solutions of $CoNO_3$ and $CuSO_4$ on the Basis of Relative Turbidity, Measured Nephelometrically*

Conc. of Salt	Salt: $CoNO_3$ — No. Days Incubated			Salt: $CuSO_4$ — No. Days Incubated		
	2	5	10	2	5	10
Control	100	100	100	100	100	100
.000005 *N*	100	105	98	99	106	99
.00001 *N*	95	119	102	98	124	97
.000025 *N*	95	114	101	106	132	102
.00005 *N*	88	107	103	119	152	129
.000075 *N*	89	105	99	116	145	110
.0001 *N*	72	95	69	91	84	98
.00025 *N*	62	46	42	87	56	64
.0005 *N*	38	33	26	82	60	45

Each figure represents an average of the readings on 9 cultures.

Summary

When a relatively heavy inoculum of *Es. coli* was introduced into broth containing .0001 *N* zinc sulfate, more gas was produced than in the control cultures.

Es. coli colonies grown in .001 *N*, .002 *N*, .003 *N*, and .004 *N* zinc sulfate agar were initially larger than the control colonies. After eight hours incubation the size difference was most pronounced. The contrast was less marked at twelve and sixteen hours and after twenty and twenty-four the once large colonies became approximately the same size as the controls. The greatest growth as shown by colony area was 58 percent in .003 *N* zinc sulfate after eight hours incubation.

Es. coli colonies grown in weaker concentrations of zinc sulfate, .0001 *N* and .00025 *N*, were initially inhibited but after sixteen and twenty hours incubation they increased in size so that after twenty-four hours they were 9 percent larger than the controls. Colonies containing more than .005 *N* zinc sulfate showed no stimulation, but an increasing toxic effect.

S. marcescens colonies grown in .002 *N*, .003 *N*, .004 *N*, and .005 *N* zinc sulfate agar were initially larger than the control colonies. This difference was most marked in the early growth stages. The greatest increase was 72 percent with the .004 *N* zinc sulfate after sixteen hours incubation. A relative decrease in the size followed, which in the case of those which had been most stimulated approached within 6 to 8 percent of the normal after forty-eight hours.

A. chroococcum cultures grown in a mannite solution containing dilute solutions of zinc sulfate showed by their turbidity, measured nephelometrically, a growth increase in the presence of .000005 *N* and .000007 *N* zinc sulfate. This was greatest, 17 percent, after five days incubation with .000007 *N* zinc sulfate. With stronger concentrations of the salt, .000009 *N* to .002 *N*, growth was initially inhibited. No growth occurred in the presence of .005 *N* zinc sulfate.

Similar cultures containing manganese sulfate showed an increased growth with .00001 *N*, .00005 *N*, .0001 *N*, and .0005 *N* after two days incubation. This growth increase, greatest with the .00005 *N* manganese sulfate after two days, was not maintained, and after fifteen days incubation the cultures appeared subnormal. No growth occurred in the presence of .01 *N* manganese sulfate.

Other test salts were too toxic in the dilutions used to give any data on growth stimulation. Their toxicity may be judged by the highest concentrations in which no growth occurred: cobalt nitrate .0001 *N*, copper sulfate .0001 *N*, nickel nitrate .00005 *N*, and cadmium nitrate .00005 *N*.

The presence of the metallic test salts in the *A. chroococcum* cultures seemed to bring about an abnormally early pigmentation of the cells and to shorten the life of the culture.

Ps. tumefaciens grown in a synthetic medium at 22° C. and 28° C. was shown by turbidity measurements to be stimulated at both temperatures by the presence of .00005 *N* zinc sulfate. The degree of stimulation, however, was greatest with the 28° C. incubation, the maximum being 20 percent after seven days.

With an increase in the available oxgyen no growth stimulation was produced by the zinc sulfate. This was also the case when the dextrose supply was increased.

Cultures of *Ps. tumefaciens* grown at 28° C. showed a maximum growth increase of 28 percent after five days incubation in the presence of .0005 *N* manganese sulfate. As with the zinc sulfate, when the oxygen tension was increased, there was no growth stimulation produced by the manganese sulfate.

Ps. tumefaciens grown at 28° C. with dilute solutions of cobalt nitrate showed a maximum growth increase of 19 percent after five days incubation in the presence of .00001 *N* dilution of the salt, and with copper sulfate a maximum growth increase after five days of 52 percent with a .00005 *N* dilution of the salt.

This work was carried on under the kindly criticism and courteous supervision of the late Dr. Herbert M. Richards.

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STUDIES ON THE SENSITIVITY OF *MIMOSA PUDICA* III. THE EFFECT OF TEMPERATURE, HUMIDITY, AND CERTAIN OTHER FACTORS UPON SEISMONIC SENSITIVITY [1]

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In the second paper of this series I have presented results of quantitative experiments on the effect of animal anesthetics and certain other compounds upon the seismonic sensitivity of *Mimosa pudica*. From the beginning of these studies it was observed that some of the commonly accepted statement in textbooks and in the literature as to the effect of certain environmental factors upon the sensitivity of the plant could not be confirmed. The present paper gives the results of experiments designed to test several of these factors specifically and to show the exact quantitative effect of each on sensitivity.

The experiments were made during the period from September 15th to March 1st. The low light intensity characteristic for this season of the year necessitated the use of artificial illumination to supplement natural illumination in order to produce healthy, vigorous plants. I have already reported in paper II the fact that plants not so treated reached a condition early in September such that they could no longer be anesthetized by ether, but that plants exposed to adequate supplementary illumination with high temperature were easily anesthetized at all times.

The plants were grown in a small room in the greenhouse which had been equipped with two 1500-watt Mazda bulbs and Benjamin reflectors. The tips of the bulbs were about 20 inches from the tops of the pots containing the plants. The light intensity, as determined by a Macbeth illuminometer, varied from 150 foot candles for the center of the field to 100 foot candles at the margin. The heat given off by the bulbs kept the temperature of the room around 20° to 25° C., but not uncommonly the temperature went much higher during the middle of the day. This high temperature is, however, very favorable for the growth of the plants. The lights were on daily from 8 A.M. to 10 P.M. The humidity in the room varied greatly, from 10 percent to 90 percent, but the plants grew equally well at both extremes.

The environmental factor studied and controlled most carefully was temperature. The plants to be tested were placed in chambers adjusted to the desired temperature and allowed to remain there for the duration of

[1] This paper is the last of a series of three appearing in successive issues of the Journal.

the experiments. The temperature control chambers in which these determinations were made were large (about a 4 foot cube) double-walled glass cases. In each case there was a three and one-half foot slow-moving turn-table upon which the plants were placed. This turn-table was in constant motion except at the time a reading was being made.

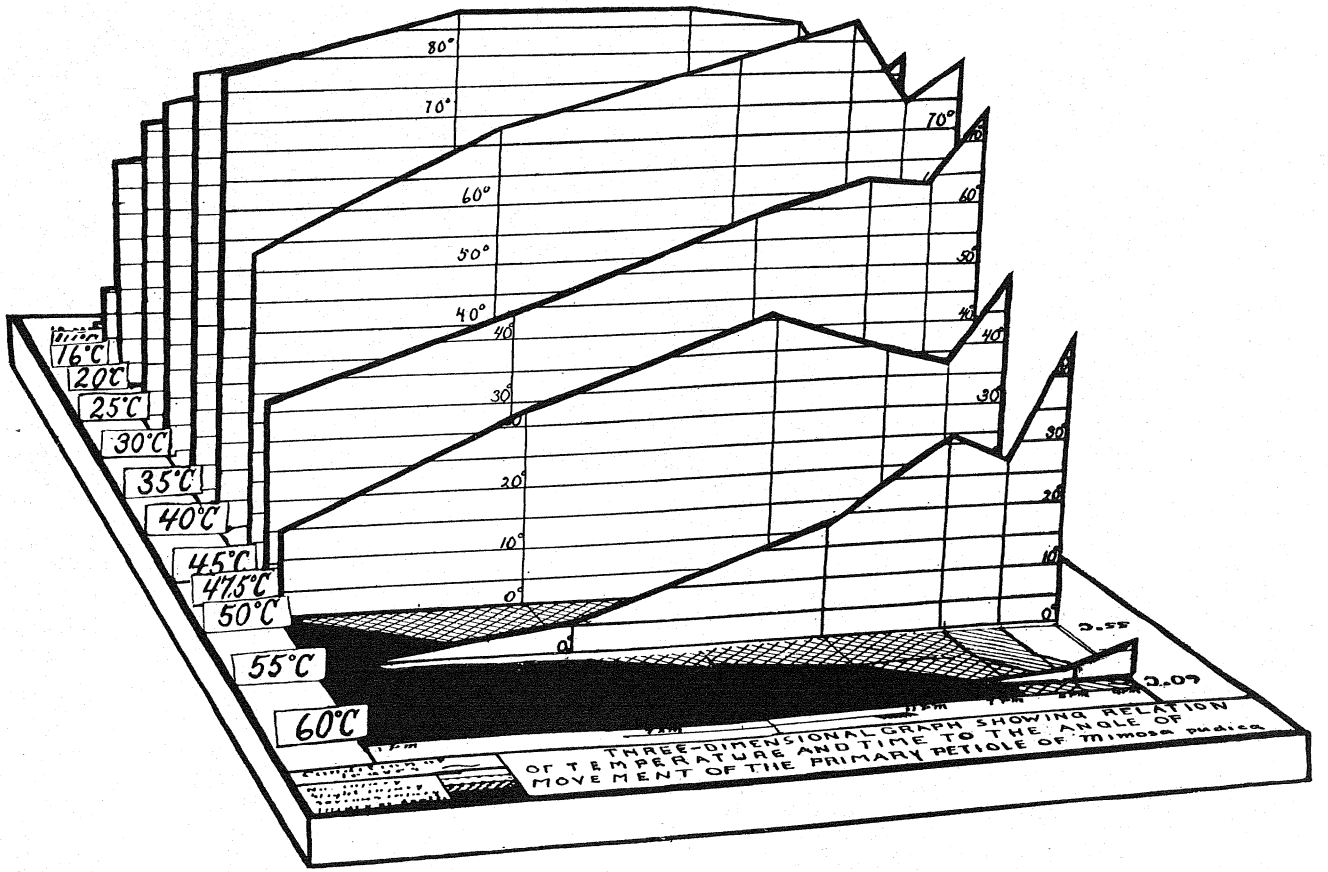
The temperature inside the chamber was maintained by one or more glow-coils, which were turned on and off by the relay and thermostat combination described by Trelease (1925). It was found that the revolution of the turn-table insured a more or less uniform temperature for any given level, but failed to maintain the same temperature at different levels. This difficulty was overcome, however, by vertical air currents set up by an electric fan placed horizontally in the back of the chamber. The rate of movement of the fan was controlled by a rheostat, so that the air current was at no time sufficient to cause movement of the leaves. This system maintained the desired temperature within .5° C. This arrangement had the additional advantage that it is easily adjusted to any temperature within the ordinary biological range. For temperatures below 25° C. a similar arrangement was used, the low temperature being secured by drawing through the experimental chamber cool air from a frigid-air machine.

The readings were made through an opening in the door of the chamber covered by a curtain. The apparatus for measuring angles (text figs. 1 and 2, second paper) was placed on the turn-table inside the chamber. The person making the reading then stood outside with his head, arms, and shoulders inside, sighted across the petiole to the protractor, and made the reading of the angle in the usual manner. As the fan and glowcoils remained on while readings were being taken, there was no appreciable change in temperature.

Temperature

I have determined the effect of the entire physiological range of temperature on the sensitivity of the primary petiole of *Mimosa pudica*. This plant remained sensitive in an extremely wide range of temperatures, from 14° to 60° C.

All points on the temperature curves except the 20° and 50° C. curves are based on the average from twenty readings on five plants, each having four mature leaves. The curves for these two exceptions are based on forty readings to each point. The plants were kept in the constant temperature chamber for a period of twenty-four hours. The readings were made one, two, four, eight, sixteen, and twenty-four hours after the set of plants was placed in the chamber. All tests began at 3 P.M. so that the readings were made at 4 P.M., 5 P.M., 7 P.M., 11 P.M., 7 A.M., and 3 P.M., respectively. Later experiments establishing the close correlation between the degree of sensitivity of *Mimosa* and the time of the day have shown how essential this original precaution was. Also the variation in sensitivity described below between the first, second, third, and fourth



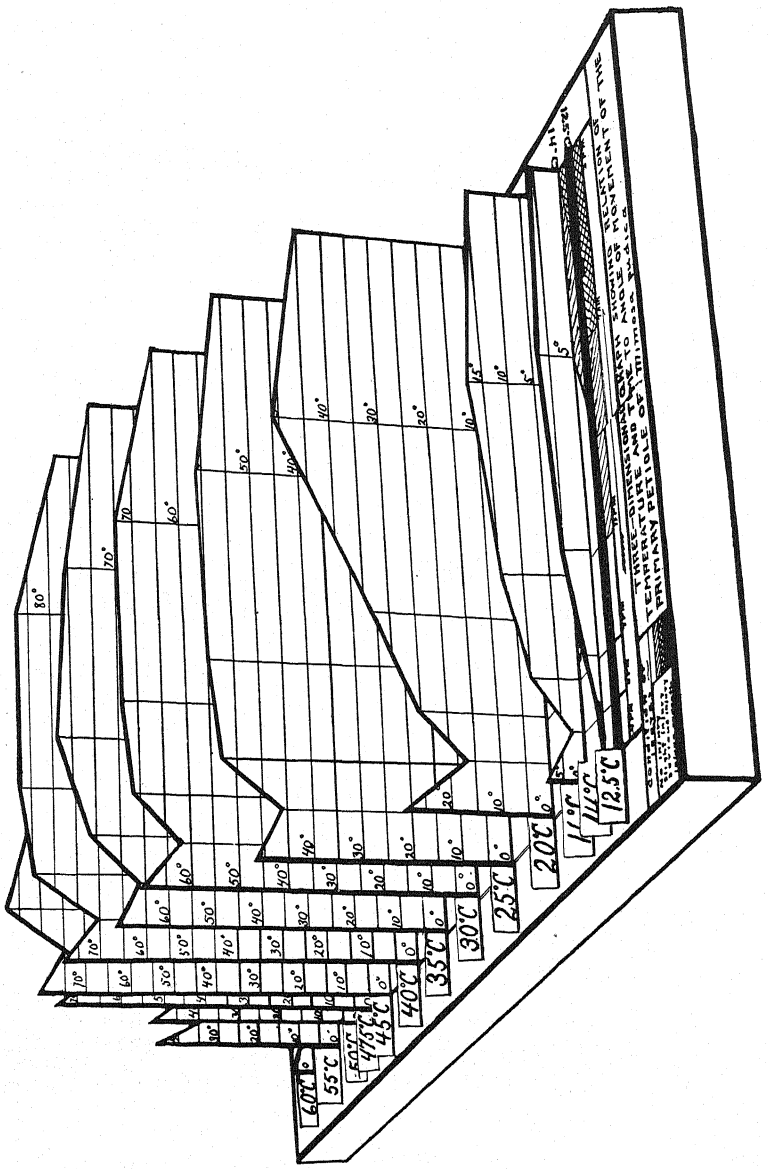
TEXT FIG. 7. Photograph taken from the high temperature end of the three-dimensional graph. All curves except two in this three-dimensional graph are based on 120 readings. Thus each point on each curve is based on the average of 20 readings. The 20° and 50° C. curves are based on just double this or 40 readings for each point. This seemed necessary for these two temperatures since the curves were different from those adjacent to them. For further details see text.

leaves shows how necessary it was to use only the first four mature leaves in making the determinations.

These determinations upon the effect of temperature on sensitivity were made from November 21st to December 3d. The fact that only three temperature control chambers were available for the studies made it impossible to run all tests at once. This distribution of the readings over a period of days was, however, probably an advantage, since it tended to eliminate many errors which might have occurred if all were run simultaneously. Also, as can be seen in table 8, only temperatures differing by ten or more degrees were tested at the same time. The determinations were made in the following order: November twenty-first, 16°, 30°, and 40° C.; November twenty-third, 20° and 45° C.; November twenty-fourth, 35° and 50° C.; November twenty-ninth, 14° and 50° C.; December first, 12.5°, 25°, and 55° C.; and December third, 20°, 47.5°, and 60° C.

In table 8 are given the data for sensitivity in the temperatures of 12.5°, 14°, 16°, 20°, 25°, 30°, 35°, 40°, 45°, 47.5°, 50°, 55°, and 60° C., respectively, for lengths of exposure ranging from one to twenty-four hours. These data for the various temperatures have shown such a close mathematical correlation that it has been possible to construct a three dimensional graph showing the relation between exposure time (also hour of day), temperature, and angle of movement. Text figures 7 and 8 are photographs of the model which was made. Figure 7 shows the high temperature end and figure 8 the low temperature end of the graph.

I have already mentioned that the 20° and 50° C. curves are based on 40 readings to each point rather than 20 as in all other temperatures. This seemed desirable because the first curves obtained for these temperatures differed quite markedly from what would be expected from the curves adjacent to them. This is especially true for the 20° C. (text figs. 7 and 8). The first four readings seem to be much too low or the last two much too high to give the typical curve. The first determinations for sensitivity at 20° C. were made on November twenty-third, and this type of curve was found. I then completed most of the determinations for other temperatures, but found no similar variation, and concluded that the first set of determinations must have been wrong. The experiment was therefore repeated on December third, nine days later, and the same results obtained. The curve that is used in the three-dimensional graph is the composite of these two series of determinations. The only explanation which I have for this atypical curve at 20° C. is that 20° C. was the approximate temperature at which the plants were grown and therefore is the only case in which there was no adjustment to a new temperature. I likewise repeated the 50° C. determinations because it seemed from the three-dimensional graph (text fig. 7) that the sensitivity at 50° C. was greater than would be expected from that at 47.5° and 55° C. The second test, however, was quite similar to the first, as can be seen in table 8.



TEXT FIG. 8. Photograph taken from the low temperature end of the three-dimensional graph. Note the variation of the 20° curve from that which is typical for all the other curves from the 40° down. The fact that this is approximately the temperature at which the plants were grown may possibly explain this discrepancy.

TABLE 8. *The Effect of Temperature on the Sensitivity of the Primary Petiole of Mimosa pudica*

Temp. ° C.	Date	Time of Day	Position of Petioles		Angle of Movement
			Initial	Final	
12.5	Dec. 1	4 P.M.	86.3	86.3	.0
		5 P.M.	106.3	106.3	.0
		7 P.M.	105.8	105.8	.0
		11 P.M.	petioles seriously injured		.0
		7 A.M.	" "	"	.0
		3 P.M.	" "	"	.0
Average for 24-hour period			99.5	99.5	.0
14.0	Nov. 29	4 P.M.	117.5	116.8	0.7
		5 P.M.	138.0	136.9	1.1
		7 P.M.	126.8	123.8	3.0
		11 P.M.	93.4	88.0	5.4
		7 A.M.	69.5	61.9	7.6
		3 P.M.	83.6	77.0	6.6
Average for 24-hour period			104.8	100.7	4.1
16.0	Nov. 21	4 P.M.	154.7	147.6	7.1
		5 P.M.	137.3	134.7	2.6
		7 P.M.	120.7	112.2	8.5
		11 P.M.	100.4	91.0	9.3
		7 A.M.	101.9	84.3	17.6
		3 P.M.	89.0	72.7	16.3
Average for 24-hour period			117.3	107.1	10.3
20.0	Dec. 3 (test no. 1)	4 P.M.	142.0	113.6	28.4
		5 P.M.	119.9	100.8	19.1
		7 P.M.	99.0	77.1	21.9
		11 P.M.	72.6	41.2	31.4
		7 A.M.	126.6	78.9	47.7
		3 P.M.	111.9	66.4	45.5
Average for 24-hour period			112.0	79.7	32.6
20.0	Nov. 23 (test no. 2)	4 P.M.	149.9	120.2	29.7
		5 P.M.	119.0	104.6	14.4
		7 P.M.	108.5	79.6	28.9
		11 P.M.	82.3	52.0	30.3
		7 A.M.	125.7	70.6	55.1
		3 P.M.	113.0	66.5	46.5
Average for 24-hour period			116.4	83.9	34.2
25.0	Dec. 1	4 P.M.	141.0	89.4	51.6
		5 P.M.	126.2	79.8	46.4
		7 P.M.	114.7	56.5	58.2
		11 P.M.	117.1	56.7	60.4
		7 A.M.	119.0	63.4	55.6
		3 P.M.	131.5	74.1	56.4
Average for 24-hour period			124.9	70.2	54.6
30.0	Nov. 21	4 P.M.	139.8	69.8	70.0
		5 P.M.	132.3	69.8	62.5
		7 P.M.	124.1	52.5	71.9
		11 P.M.	140.1	67.0	73.1
		7 A.M.	139.1	65.5	73.6
		3 P.M.	135.2	70.3	64.9
Average for 24-hour period			135.1	65.8	69.3

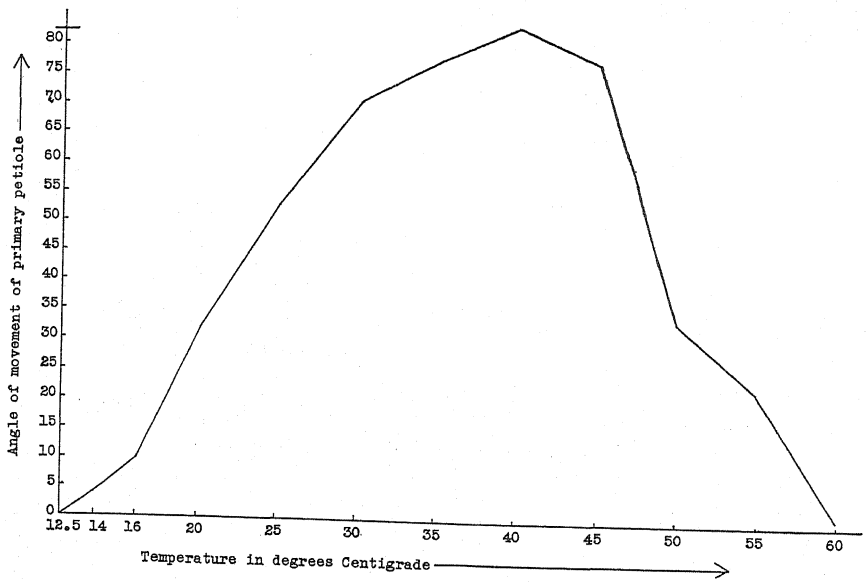
Each value given in this table for initial and final position, etc., is the average obtained from twenty primary petioles on five different plants. A new set of five plants was used for each trial for each temperature.

TABLE 8—*Continued*

Temp. °C.	Date	Time of Day	Position of Petioles		Angle of Movement
			Initial	Final	
35.0	Nov. 24	4 P.M.	129.9	58.1	71.8
		5 P.M.	128.6	62.0	66.6
		7 P.M.	136.1	59.9	76.2
		11 P.M.	152.1	68.5	83.6
		7 A.M.	139.6	58.6	81.0
		3 P.M.	139.1	63.7	75.4
Average for 24-hour period			137.6	61.8	76.3
40.0	Nov. 21	4 P.M.	133.4	54.9	78.5
		5 P.M.	131.1	59.6	71.5
		7 P.M.	145.7	60.5	85.2
		11 P.M.	154.9	66.7	88.2
		7 A.M.	151.8	65.9	85.9
		3 P.M.	140.1	61.8	78.3
Average for 24-hour period			143.3	61.6	81.3
45.0	Nov. 23	4 P.M.	146.1	64.0	82.1
		5 P.M.	152.6	77.3	75.3
		7 P.M.	157.4	69.0	88.4
		11 P.M.	159.6	76.4	83.2
		7 A.M.	155.9	83.6	72.3
		3 P.M.	139.3	86.5	52.8
Average for 24-hour period			151.7	76.1	75.7
47.5	Dec. 3	4 P.M.	146.2	70.6	75.6
		5 P.M.	148.4	84.9	63.5
		7 P.M.	137.2	72.5	64.7
		11 P.M.	140.3	81.4	58.9
		7 A.M.	136.7	92.1	44.6
		3 P.M.	138.1	96.6	31.5
Average for 24-hour period			141.2	83.0	56.5
50.0	Nov. 24 (test no. 1)	4 P.M.	129.9	76.2	53.7
		5 P.M.	134.8	90.4	44.4
		7 P.M.	133.0	93.0	40.0
		11 P.M.	156.0	114.9	41.1
		7 A.M.	141.2	106.9	34.3
		3 P.M.	137.0	121.8	15.2
Average for 24-hour period			138.7	105.3	38.1
50.0	Nov. 29 (test no. 2)	4 P.M.	141.1	98.7	42.4
		5 P.M.	150.3	121.3	29.0
		7 P.M.	148.8	110.5	38.3
		11 P.M.	141.6	91.3	50.3
		7 A.M.	136.5	106.1	30.4
		3 P.M.	129.1	115.1	14.0
Average for 24-hour period			142.9	107.3	34.1
55.0	Dec. 1	4 P.M.	172.5	123.8	48.7
		5 P.M.	131.0	103.5	27.5
		7 P.M.	129.1	98.2	30.7
		11 P.M.	118.7	100.0	18.7
		7 A.M.	73.8	71.0	2.8
		3 P.M.	petioles almost dead		.0
Average for 24-hour period			125.0	99.3	21.6
60.0	Dec. 3	4 P.M.	94.4	88.7	5.7
		5 P.M.	88.3	86.5	1.8
		7 P.M.	petioles apparently dead		.0
		11 P.M.	" " "		.0
		7 A.M.	" " "		.0
		3 P.M.	" " "		.0
Average for 24-hour period					

The point which stands out above all others in this study is the great range of air temperatures over which *Mimosa* retains its sensitivity. Fourteen degrees centigrade is the minimum temperature at which I have found movement, and the plant continues to show movement in temperatures up to 60° C. This latter temperature is, of course, slightly higher than the thermal coagulation point of egg albumen.

The data of table 8 are plotted in text figure 9 to show the average angle of movement for each temperature studied. From 12.5° to 30° C. the curve for increased sensitivity is almost a straight line function. Above this it flattens slightly, reaching the maximum of 81.9 degrees at a temperature of 40° C. The plants which had been exposed to 40° C. for twenty-four hours were entirely normal. This is shown by the curve (text fig. 8), which is identical in shape with those for 25°, 30° and 35° C. Moreover the sen-



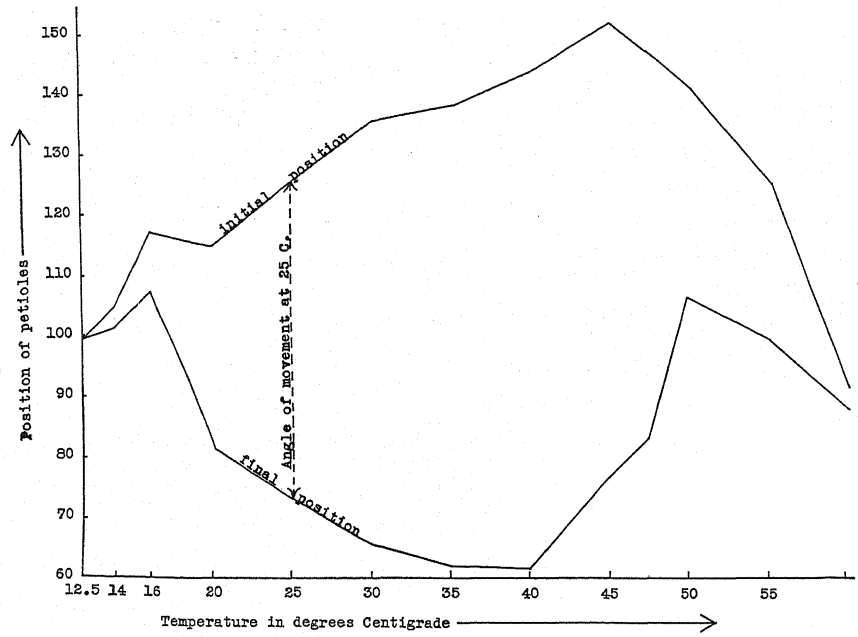
TEXT FIG. 9. The average effect of temperature over a twenty-four hour period upon the sensitivity of the primary petiole of *Mimosa pudica*. Data from table 8.

sitivity after twenty-four hours differs by less than one degree from what it was initially (table 8), showing that no injury had occurred.

Plants in a temperature of 45° C. gave an angle of movement for the first few hours which was greater than that at 40° C., but the curve then fell off rapidly (text fig. 7), due apparently to injury resulting from the longer exposure. The amount of movement decreases rapidly with still higher temperatures, the plants being almost insensitive at 60° C.

The increase in sensitivity, with increase in temperature up to the optimum, 40° C., is in general expressed in a combination of two factors:

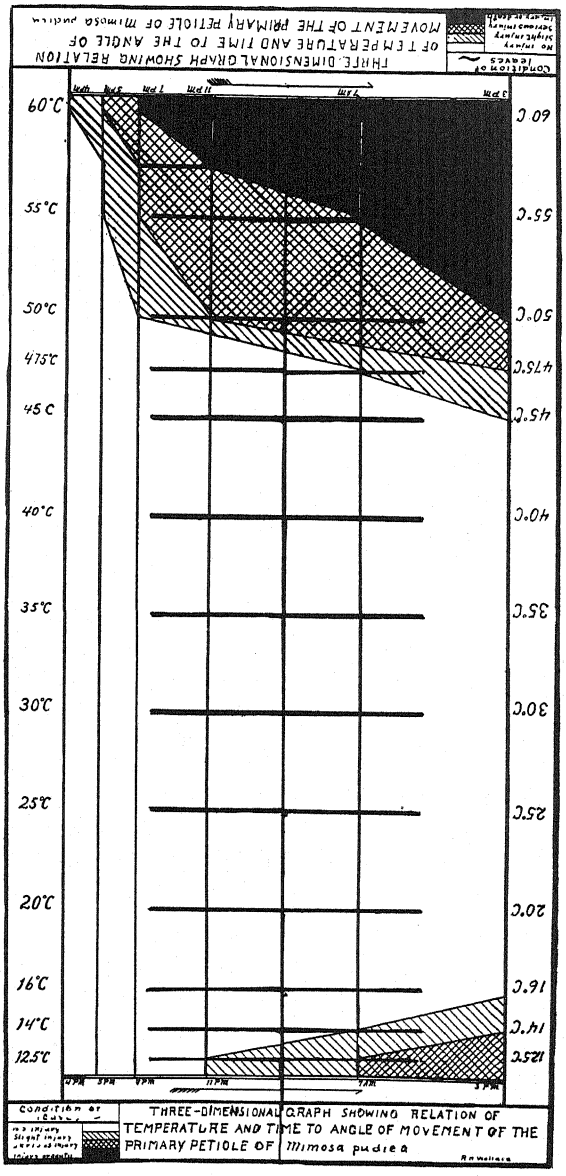
a progressively higher position of the petiole before stimulation, and a progressively lower position of the petiole after stimulation. This is shown in text figure 10 (data from table 6) in which the upper curve represents the average "initial" position, and the lower the average "final" position of the petioles. The distance between the two curves for any given tempera-



TEXT FIG. 10. Curves showing the average initial and final petiole position for the various temperatures tested. Data from table 8.

ture is of course the angle of movement, or sensitivity for that temperature. There is an increase in the angles for both the "initial" and "final" positions when the temperature is raised from 12.5° to 14° and 16° C., but the curves then both break at 20° C. It is interesting to note that this break occurs at the only temperature, below the optimum, which gives an atypical curve. The angle of "final" position continues to decrease until it reaches a minimum of 62 degrees at 40° C. The "initial" position on the other hand increases rapidly in temperatures above 20° C., reaching a maximum elevation of 152 degrees at a temperature of 5° C. above the optimum temperature. It then breaks abruptly and decreases ever more rapidly until the angle is only 92 degrees at 60° C. The "final" position of the petioles tends to approach its original value (an angle of 100 degrees at 12.5° C.) at 50° C., but the curve then breaks abruptly, due apparently to the serious injury which occurs at temperatures of 55° and 60° C.

The temperatures at either extreme that cause the plants to be insen-



TEXT FIG. 11. Photograph of the ground plan of the three-dimensional graph showing the qualitative effects of the various temperatures upon the leaves of the plants. White, no injury; single-hatched, slight injury; double hatched, serious injury; black, severe injury or death.

sitive or almost so will generally cause serious injury or death within a few hours. This is shown in text figure 11 which is a photograph of the ground plan of the three-dimensional graph. It will be seen that an exposure of eight hours to 12.5° C. resulted in slight injury while a sixteen-hour exposure to the same temperature resulted in serious injury. At 14° C., however, an exposure of sixteen hours was required to produce slight injury, and an exposure of twenty-four hours was required at 16° C. to give the same effect.

Plants were very seriously injured or killed after exposures of four hours at 60° C., sixteen hours at 55° C., or twenty-four hours at 50° C. They were seriously injured after two hours at 60° C., four hours at 55° C., eight hours at 50° C., or sixteen hours at 47.5° C. They were slightly injured after one hour at 60° C., four hours at 55° C., eight hours at 50° C., sixteen hours at 47.5° C., or twenty-four hours at 45° C. They were entirely uninjured after two hours at 55° C., four hours at 50° C., eight hours at 47.5° C., sixteen hours at 45° C., and twenty-four hours at 40° C.

The injury in both the high and low temperatures appeared first as water deficiency in the plants; that is, the leaves of the plants, whether at 12.5° or 60° C., seemed to wilt and dry up. In the case of the low temperatures this injury was seldom severe, and the plants appeared almost normal a few days after the experiments. In the high temperatures, however, the plants were often seriously injured or killed. A shorter exposure to high temperatures resulted in a slight permanent injury and a still shorter exposure in a slight curling which entirely disappeared in a day or so. It might be well to state here that it was found necessary to place the pots in saucers full of water when experiments were made above 30° C. to prevent injury from drought.

Sachs (1863), Pfeffer (1906), and others have reported heat and cold rigor for *Mimosa*. I have been unable to bring about this condition without causing injury. It is true that the plants become insensitive when exposed to a temperature of 12.5° C., but it will also be observed in text figure 11 that a sixteen-hour exposure to this temperature caused serious injury. Cold rigor, therefore, can be said to exist only in temperatures which injure.

Heat rigor, on the other hand, does not exist even in temperatures which kill. The plants have remained sensitive in a temperature of 60° C. for two hours even though a four-hour exposure to this temperature resulted in the death of all leaves. Perhaps the most striking point in connection with injury by high temperatures was that the primary pulvini often remained sensitive after the leaflets and even part of the primary petiole were entirely dead and dried up.

There is, however, a phenomenon which can easily be mistaken for heat or cold rigor. When a plant which has become adjusted to a given temperature is suddenly transferred to a temperature several degrees higher or lower than the initial temperature, the plant may become entirely insensitive

for a short time (5 to 15 minutes). It then regains its sensitivity and shows the angle of movement characteristic for the new temperature. This transitory loss of sensitivity is more conspicuous in high temperatures. Also it seems that plants which have been kept at constant temperature for several days may show this temporary loss of sensitivity with an alteration of only two or three degrees in temperature.

Humidity

All experiments which I have made show that the relative humidity of the environment has little or no visible effect upon the sensitivity of *Mimosa*. The data in table 8 upon the effect of various temperatures on sensitivity were obtained in humidities ranging from 4 percent to about 35 percent, and it is quite obvious that, if humidity were an important factor, this rather broad range would have made impossible the close mathematical agreement in these data. Nevertheless an experiment was run to test specifically the effect of humidity on sensitivity. Two of the chambers already described were adjusted so as to maintain a temperature of 30° C. One was maintained just as dry as possible (about 10 percent humidity) and the other was humidified by allowing steam to escape inside (about 95 percent humidity). In spite of this very great difference in relative humidity the two sets of plants gave angles of movement of 79.2 and 73.5, respectively, thus indicating perhaps a slightly decreased sensitivity for the very high humidity.

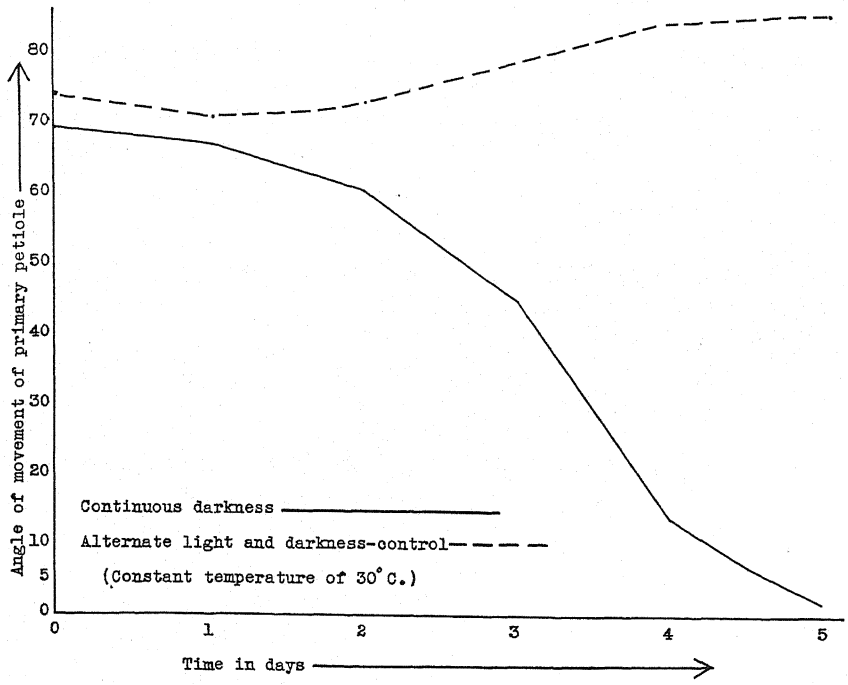
Darkness

It is well known that *Mimosa* becomes pathological and loses its leaves when kept in weak light. Also Pfeffer (1906) reports that the plants become entirely insensitive after four or five days in darkness. I made a series of tests to determine the sensitivity after various lengths of time in total darkness, and obtained results similar to those of Pfeffer.

These tests were made in two of the control chambers adjusted to maintain a temperature of 30° C. One chamber was converted into a dark chamber by surrounding the glass case with wall-board, while the other remained as in the experiments on temperature. Five plants each with four mature leaves were placed in the two chambers and readings made 1, 2, 3, 4, and 5 days after enclosure. In text figure 12 are given the curves for the two sets of plants. Plants *A* in the diffused light of the control chamber retained their usual sensitivity for the five day period. Plants *B*, however, in continuous darkness showed a very rapid loss of sensitivity. The first day the angle of movement was just 2.5 degrees less than at the beginning of the test. It then dropped 5, 15, 30, and 13 degrees successively on the days following until the angle of movement at the end of the fifth day was only 2.2 degrees.

The loss of sensitivity in continuous darkness seems to be due to starva-

tion of the leaves, since they deteriorated rapidly and most of them were entirely dead at the close of the experiment. The control plants, on the contrary, appeared as fresh and vigorous as when placed in the chamber. It will be observed in table 9 that the petioles of the plants in continuous darkness were elevated about 30 degrees above normal at the end of the



TEXT FIG. 12. The effect of light and darkness on the sensitivity of the primary petiole of *Mimosa pudica*. Data from table 9.

TABLE 9. *The Effect of Light and Darkness on the Sensitivity of the Primary Petioles of Mimosa pudica*

Length of Exposure	Alternate Light and Darkness			Continuous Darkness		
	Position of Petioles		Angle of Movement	Position of Petioles		Angle of Movement
	Initial	Final		Initial	Final	
1 hour.....	137.4	63.7	73.7	141.6	72.9	68.7
1 day.....	126.0	56.5	70.6	140.3	73.6	66.7
2 days.....	152.5	80.6	71.6	163.5	103.6	60.2
3 days.....				174.5	129.9	44.6
4 days.....	140.6	65.6	75.0	147.4	133.6	13.8
5 days.....	142.0	70.0	72.0	120.6	113.7	6.9

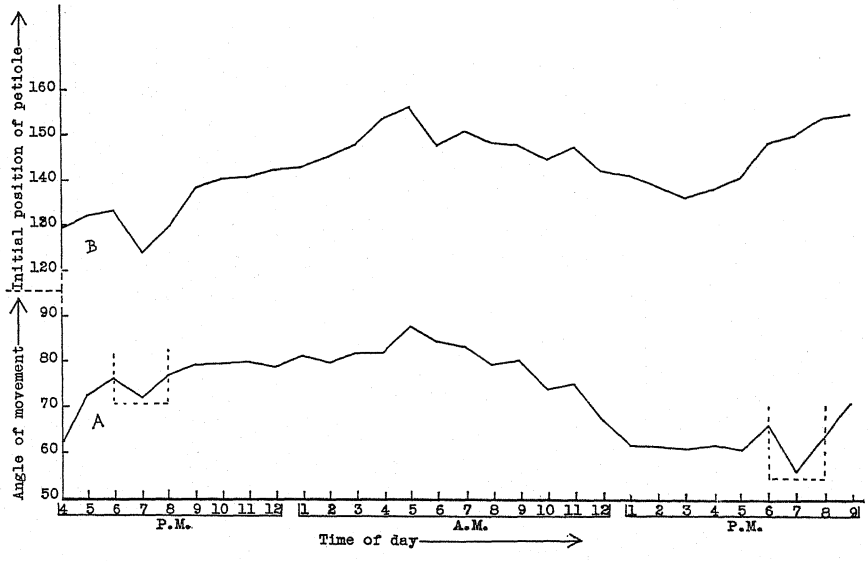
Experiment begun January 29, 3 P.M.; approximate humidity 20 percent; constant temperature chamber adjusted for 30° C.

third day and then became depressed below normal during the last two days of the experiment. This initial elevation of petioles when light is decreased or absent will be noted in connection with observations on daily periodicity.

Daily Periodicity

There is a very marked variation in the sensitivity of *Mimosa* at different times of day. This fluctuation was observed in the experiments in the temperature studies. Thus it will be noted (text figs. 7 and 8), that the 16°, 30°, and 40° C. curves, the data for which were obtained the first day, all show an abrupt drop at 5 P.M. It seemed at first that this sudden drop might be due to a readjustment of the plant to new temperatures, but this was rendered doubtful by the wide range of temperatures tested the first day; and when later tests showed the same drop in all temperatures except the extremes (which were injurious), it was quite obvious that temperature adjustment was not the explanation. Moreover, the fact that the curve for 20° C., the approximate temperature at which the plants were grown, showed the same effect indicated strongly that daily periodicity must be the cause of the drop.

In order to determine whether the variation in sensitivity was due to daily periodicity a set of plants was tested hourly for thirty hours. These



Text Fig. 13. The fluctuation in sensitivity and initial position of petioles for various times of day. These two curves show a mathematical correlation of .70. Data from table 10.

tests were made at a constant temperature of 30° C. from 4 P.M., February 2, to 9 P.M., February 3, just two months after the temperature studies had been completed. The curve obtained, *A* in text figure 13, is similar to

those in the three-dimensional graph for temperatures ranging from 16° to 40° C., inclusive. The abrupt evening drop occurred now, however, at 7 P.M., rather than at 5 P.M. as it had two months earlier. It will be seen that this drop is repeated at 7 P.M. the second day, as would be expected if it were due to daily periodicity. The maximum sensitivity of *Mimosa* seemed in this test to be at 5 A.M., but it is impossible to know whether there was a like shift in the time of maximum sensitivity since the data shown in the three-dimensional graph were for eight-hour intervals during this period of the day.

I have noted above that a progressively higher "initial" and a progressively lower "final" position of the petiole accounted for the increase in sensitivity as the temperature was raised from 20° to 40° C. This combination of a higher "initial" and a lower "final" position does not, however, explain the variation in sensitivity with time of day. Here, as can be seen in text figure 11, the fluctuation in sensitivity is due primarily to variations in the "initial" positions of the petioles. Statistical analysis of these data shows a coefficient of correlation of .70 between initial position and angle of movement, while that between final position and angle of movement was only .20. It is obvious from these values that final position played but a minor role in the test on daily periodicity.

These calculations of coefficients of correlation are based on the readings for only the first twenty-four hours of the test and not on the entire series. This was necessary because the second day of the test, February 3, was a very dark day and caused an elevation of petioles evident at the right end of the curve. I have already noted this effect of darkness on petiole position in the section on the effect of darkness. This very poor light for the second day of the experiment also probably explains the fact that the position of the petiole at 4 P.M. on this day was 7 degrees higher than it was twenty-four hours earlier when the test began. If correction were made for this elevation of 7 degrees above normal due to the effect of weak light, the coefficient of correlation between "initial" position and angle of movement would be much higher. It is interesting to note that the abrupt drop in sensitivity mentioned above as occurring at 7 P.M., the second day, occurred even though the petioles were becoming progressively higher at this time.

Age of Leaf

I have already mentioned that all determinations in this paper, as in the preceding one on the effect of animal anesthetics and certain other compounds on sensitivity of *Mimosa*, have been made on just the first four mature leaves. This precaution was observed because it seemed quite possible that sensitivity might vary with the age of the leaf. It will be recalled that the plants were usually not more than six weeks old. Under these conditions of growth there was never more than three weeks difference in age between leaf number one, the youngest, and leaf number four, the

oldest used. In fact there was often a difference of only two weeks or less in their ages, because the plants when growing best may produce four leaves a week.

TABLE 10. *Diurnal Periodicity in the Sensitivity of the Primary Petioles of Mimosa pudica*

Time of Day	Position of Petiole		Angle of Movement in Degrees
	Initial	Final	
4 P.M.	129.8	67.0	62.8
5 P.M.	132.3	59.8	72.5
6 P.M.	133.3	56.6	76.7
7 P.M.	123.9	51.3	72.6
8 P.M.	129.7	53.0	76.7
9 P.M.	137.9	58.4	79.5
10 P.M.	139.6	59.9	79.7
11 P.M.	140.2	60.4	79.8
12 P.M.	141.9	62.9	79.0
1 A.M.	142.4	61.4	81.0
2 A.M.	143.3	63.8	79.5
3 A.M.	147.9	66.1	81.8
4 A.M.	152.9	71.1	81.8
5 A.M.	157.3	69.5	87.8
6 A.M.	147.3	62.9	84.4
7 A.M.	151.1	67.9	83.2
8 A.M.	148.5	69.3	79.2
9 A.M.	147.9	67.7	80.2
10 A.M.	144.7	71.2	73.5
11 A.M.	147.4	72.1	75.3
12 A.M.	142.4	76.6	65.8
1 P.M.	139.7	78.1	61.6
2 P.M.	137.5	75.5	62.0
3 P.M.	136.9	75.7	61.2
4 P.M.	138.6	76.6	62.0
5 P.M.	140.8	79.5	61.3
6 P.M.	148.5	80.6	66.4
7 P.M.	151.9	95.3	56.6
8 P.M.	153.9	90.7	63.2
9 P.M.	155.3	89.5	70.8

Experiment begun February 2, 3 P.M. in a chamber adjusted to maintain a constant temperature of 31.5° C. Each value given for initial and final position, etc. is the average from twenty petioles on five plants.

Table 11 gives the same data as that given in table 10 on daily periodicity except that it is divided on the basis of the age of leaf. This set of five plants was tested hourly for thirty hours and is the largest continuous series of tests which I have on a single set of plants. The total number of readings for the series is 600, or 150 for each age of leaf. It will be observed that leaf number one gave an average angle of movement of 81.5 degrees; leaf number two, 79.3; leaf number three, 74.3 and leaf number four, 67.9. There is, therefore, a very pronounced decrease in sensitivity with slight increased age of leaf.

The use of leaves of the same age was of fundamental importance, espe-

TABLE 11. *The Relative Sensitivity of Petioles of Different Ages*

Number of Leaf	Position of Petiole		Angle of Movement
	Initial	Final	
1	140.4	58.9	81.5
2	139.6	60.3	79.3
3	139.6	65.3	74.3
4	145.8	77.9	67.9

The youngest mature leaf was taken as number 1 while the oldest leaf tested was number 4.

The data here tabulated are the same as those given in table 10 on daily periodicity except that they are arranged according to age of leaf. Therefore, each value given for initial and final position, etc., is the average obtained from 120 petioles.

cially in the temperature studies. Thus the average angle of movement of leaves one, two, three, and four given above was 75.7 degrees. If one calculates the average angle of movement for leaves two, three, four, and five (allowing a decrease of 1.5 degrees for leaf number five below that for leaf number four) a value of only 70.5 degrees is obtained. This difference is almost equal to the increase in sensitivity between temperatures of 30° and 35° C. or 35° and 40° C.

DISCUSSION

The first serious attempt to determine the effect of temperature on the sensitivity of *Mimosa pudica* is that of Sachs (1863). Pfeffer (1906) has summarized this work in his text book of plant physiology in the following manner. "Sachs found transitory heat rigor was produced by exposure to 40° C. for an hour, at 45° C. in half an hour and at 49° C. to 50° C. in a very brief time. In some cases plants which were still irritable at 40° C. became transitorily rigid when brought to a normal temperature, either as the after-effect of the previous exposure or owing to the shock effect of the sudden change."

This work of Sachs was of course made by means of the crude temperature control methods available at that time, but even so it is difficult to understand how loss of sensitivity could have been produced at such relatively low temperatures. This is especially true in view of the fact that Bert only nine years later, 1870, reports temperature effects which are easily verified today. I can perhaps summarize my own work in no better manner than by quoting Bert's entire description of temperature effects. He says:

> I have made experiments to determine the degree of elevation of temperature which a sensitive plant would be able to stand without losing its sensitivity, or having lost its sensitivity, without dying. I will say that on every occasion that sensitivity has been definitely destroyed, I have always observed that the plant died. But sensitivity can disappear to reappear later (Julius Sachs). The temperatures endured by my sensitive plants have been much higher than the temperature indicated as the upper limit by Julius Sachs (52° C.).

Thus on September 6, a sensitive plant was placed in sunlight under a belljar at 9:05 (a.m.); at 9:50 (a.m.) the temperature of the air is 47°: the leaves two and three give me 63° and 70° of fall on stimulation. At 10:10 the plant left in diffused light is returned to sunlight. At 10:45 the temperature is 51°; stimulation gives falls of 83° and 57°. At this high temperature the leaflets are slightly closed.

I have even seen a sensitive plant remain sensitive in a high humidity where the temperature taken near the level of the soil in the pot had risen in seventeen minutes from 28° to 55°, and in the eight minutes following from 56° to 62°.

As will be noted, Bert records sensitivity at a temperature two degrees higher than I have tested in my studies. This temperature of 62° C. is extremely high, in terms of most biological processes, and must result in serious injury to the plant in a relatively short time. If heat rigor for *Mimosa pudica* exists at all one would expect to find it here. I am sure in my studies that any loss of sensitivity which could possibly be termed heat rigor represents such a transitory state between loss of sensitivity and death as to be of no consequence. In like manner the so-called dark rigor for *Mimosa* which Sachs and others have reported seems in my studies to be lacking or so closely associated with injury and death as to be meaningless. I have some evidence of a loss of sensitivity over a narrow range of low temperature where the term cold rigor could be used. It might be well to state while on the subject of rigor that I have never found drought rigor. Many plants have been allowed to dry up and die after they had been used for various tests in this series of papers. I have, however, never found plants to be insensitive except after they had been seriously injured by the drought.

It has been mentioned above that there was marked variation in sensitivity with time of day and I have suggested that this seemed to be due chiefly to fluctuation in "initial" petiole position. In general the sensitivity tends to become progressively greater from early evening until early morning (about 5 A.M.). It then gradually decreases and becomes least during the period from noon until 5 to 7 P.M. Attention, however, has not been called to the fact that sensitivity becomes low soon after noon and then tends to become relatively much greater for a very brief time after which it drops abruptly and then gradually returns to the sensitivity characteristic for early evening.

I was inclined to look upon this partial recovery and subsequent drop in sensitivity which shows so conspicuously in the curve for daily periodicity as being more or less a chance result, even though the possibility of it being more fundamental was strongly suggested by its universal appearance over such a wide range of temperature in my studies.

In reviewing the literature on *Mimosa*, however, I have found good evidence in the paper of Bert that "initial" petiole position gives a curve similar to those which I have given in text figure 13 for the daily fluctuation in sensitivity and "initial" position. Bert's two curves show minimum elevation from about noon until evening, and in almost every case exhibit

the peculiar slight elevation and abrupt drop which I have already described for this period of the day. If, as is indicated by the coefficient of correlation of .70, "initial" position determines to a large extent sensitivity the two curves should be very similar.

It is very interesting to note also that Bert found an elevation of petioles during the first two days the plants were in darkness, and that they then became progressively more and more depressed during the remainder of the experiment. Reference to table 9 will show that this is the same condition which I have found. He also reports that the leaves were almost dead at the close of the experiment.

Summary III

16. A series of experiments testing the effect of certain external and internal factors upon the sensitivity of *Mimosa* have been made using materials and methods similar to those reported in papers I and II.

17. The effect of the entire physiological range of temperature from 12.5° to 60° C. has been determined for exposure times ranging from one to twenty-four hours. As the tests were begun the same time each day the data likewise show the effect of various temperatures on the sensitivity of the plant for different times of day.

18. These temperature data showed such close mathematical correlation that it was possible to construct a three-dimensional graph illustrating the relation between length of exposure (or time of day), temperature, and angle of movement.

19. The optimum temperature was found to be at 40° C.; the minimum at 14° C., and the maximum near 60° C. A temperature of 45° C. gave an angle of movement for the first few hours which was greater than that at 40° C., but the sensitivity decreased abruptly after four hours exposure due to injury from long exposure.

20. Even though temperatures of 45° C. or above caused injury or death depending upon the length of exposure, heat rigor was never observed. The plants did often, however, become transitorily insensitive when transferred suddenly to temperatures higher or lower than those to which they were adjusted.

21. Sensitivity seemed to be almost independent of the relative humidity of the environment.

22. Plants became practically insensitive after exposure to continuous darkness for four or five days, due apparently to serious injury or death of the leaves.

23. The sensitivity of *Mimosa* showed a marked diurnal variation. The period of greatest sensitivity was near 5 A.M. while the period of least sensitivity was 1 to 7 P.M.

24. The increase in sensitivity as the temperature is raised from 20° C. to the optimum, 40° C., was due apparently to a combination of a higher

"initial" and a lower "final" position of petioles, whereas the fluctuation in sensitivity with time of day seems to be correlated with variations in "initial" position.

25. The sensitivity of the leaves of *Mimosa* decreased rapidly with increasing age. Thus the average angle of movement based on 150 tests each for leaves 1, 2, 3, and 4 was 81.5, 79.3, 74.3, and 67.9 degrees, respectively.

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STUDIES IN ONAGRACEAE[1] VI. THE SUBGENUS ANOGRA OF THE GENUS *OENOTHERA*

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In this paper there are included in Anogra the plants generally so placed, except for the two species *Oenothera coronopifolia* T. & G. and *Oe. albicaulis* Pursh (*Oe. pinnatifida* Nutt.). In spite of the protest of Sprague and Riley (Kew Bull. **1921**: 198. 1922), I agree with Rose (Contr. U. S. Nat. Herb. **8**: 330. 1905) that the affinity of *Oe. coronopifolia* is with Raimannia. *Oe. albicaulis* must go there too. The white flowers and drooping buds of these two species are like those of Anogra, but the biseriate ovules, distinctly pitted subcylindric yellowish seeds, and the oblong-fusiform capsule are very near those of *Oenothera laciniata*, a Raimannia. In fact, herbarium material of *Oe. laciniata* var. *grandiflora* and of *Oe. albicaulis* can sometimes be distinguished with certainty only by the more pointed buds and free calyx-tips of the former.

In this study I have had the privilege of working up the material in the herbaria of the following institutions: University of California (C), Gray Herbarium (G), Pomona College (P), Stanford University (S), and United States National Herbarium (US). The abbreviations above indicated are those used in citing specimens. To those who kindly loaned me material and to Dr. B. L. Robinson for the privilege of the use of the herbarium and library at the Gray Herbarium, I hereby express my appreciation.

ANOGRA

Subgenus **Anogra** (Spach) Jepson, Man. Fl. Pls. Calif., 680. 1925.

Anogra, as genus, Spach, Nouv. Ann. Mus. Paris **4**: 339. 1835; Raimann, in Engl. & Prantl, Die Nat. Pflanzenfam. III, Abt. **7**: 214. 1898. As sect., Endlicher, Gen. Pl., 1190. 1840. *Baumannia*, as genus, Spach, Hist. Veg. Phan. **4**: 351. 1835.

Annual to perennial, caulescent herbs. Leaves subentire to pinnatifid. Buds nodding. Flowers vespertine, white, becoming reddish; stamens sub-

[1] No. V of this series appeared in Amer. Jour. Bot. **17**: 358–370. 1930.

[The Journal for April (**18**: 237–307) was issued April 15, 1931.]

equal. Stigma with 4 linear lobes. Capsule cylindric, slightly narrowed toward apex, sessile. Seeds in one row in each cell, linear-obovoid to obovoid, quite smooth, except under great magnification.

Type species, *Oenothera pallida* Lindl.

KEY TO SPECIES

Spring or winter annuals, coarse; basal leaf-blades rhombic, 2–8 cm. long; capsules woody, with exfoliating epidermis and 2–7 cm. long; seeds 1.5–2 mm. long; buds often shaggy. Eastern Washington south to Lower California and east to Arizona and Nevada..... 1. *Oe. deltoides.*

Perennial, perhaps occasionally biennial; basal leaves smaller and more narrow; capsules not woody (except in no. 2).

Upper portion of plant conspicuously pilose with long spreading hairs (see also *Oe. runcinata* var. *leucotricha* and sometimes *Oe. californica*). Southern half of New Mexico and adjacent Texas.

Capsules at right angles to stem, woody, 2.5–4.5 cm. long; petals 1–1.5 cm. long; leaves quite sessile.. 2. *Oe. Engelmanni.*

Capsules quite erect, not woody, 2–3 cm. long; petals 2–3 cm. long; lower leaves, at least, petioled..... 3. *Oe. neomexicana.*

Upper parts of plant not conspicuously pilose with long spreading hairs, or if so, also pallid with fine appressed hairs.

Plants essentially glabrous, though they may have a few appressed hairs, as well as fine glandular pubescence in upper parts.

Inflorescence glandular-pubescent; stems erect, 4–10 dm. tall; leaf-blades oblong-linear to oblong-lanceolate, subentire, strigose beneath. East of Rocky Mts. from Alberta to Colorado. 4. *Oe. Nuttallii.*

Inflorescence not glandular-pubescent.

Capsules often contorted; seeds linear-obovoid; plants 2–5 dm. tall, usually with lateral stems widely divaricate; leaves lanceolate to lance-linear, undulate, largely glabrous. Eastern Washington and Oregon to Utah, northern Arizona and San Juan Co., New Mexico..... 5. *Oe. pallida.*

Capsules usually not contorted.

Capsule 1–1.5 mm. thick at base; leaves deeply sinuate-dentate; seeds linear-obovoid. Southern New Mexico..... 8*b*. *Oe. runcinata* var. *brevifolia.*

Capsule 2.5–3 mm. thick at base; leaves subentire or shallowly dentate; seeds plump. Southern coastal California.. 9*b*. *Oe. californica* var. *glabrata.*

Plants canescent to hoary, strigulose or villous, especially in upper parts.

Capsules contorted; buds with long hairs as well as short appressed ones; petals 10–18 mm. long.
Leaves not deeply sinuate-dentate; foliage quite pallid; calyx-lobes with free tips. Near St. Anthony, Idaho..... 5*b*. *Oe. pallida* var. *idahoensis*.
Leaves greenish, usually sinuate-dentate; stems reddish; calyx-lobes without free tips in bud. Southwestern Wyoming and western Colorado to eastern Utah..... 6. *Oe. trichocalyx*.
Capsules spreading, usually not contorted; petals 1–3 cm. long.
Capsule rather slender, not over 2 mm. in diameter; seeds narrowly obovoid; pubescence usually fine and appressed without much admixture of longer hairs. Utah and eastern Arizona to Dakota and Oklahoma.
Leaf-blades ovate to oblong-lanceolate, entire or shallowly dentate. Dakota to Oklahoma, Colorado and Utah.... 7. *Oe. latifolia*.
Leaf-blades lanceolate, deeply dentate, or, if broader and more shallowly toothed, then usually not over 1 cm. long. New Mexico and Arizona to western Texas and Chihuahua..... 8. *Oe. runcinata*.
Capsule thicker, ca. 3 mm. in diameter; seeds plump and obovoid; pubescence with long hairs as well as the fine ones. Nevada and California..... 9. *Oe. californica*.

Treatment of Species

1. Oenothera deltoides Torr. & Frem., Rep., 315. 1845.

Oe. trichocalyx of authors for material west of Utah, not *Oe. trichocalyx* Nutt.

Coarse spring or winter annuals, simple or more frequently with centra erect stems 0.5–2.5 dm. high, and a few to several decumbent basal branches, these naked at base, 0.5–3 (10) dm. long and with exfoliating epidermis; the whole plant somewhat pale green, glabrous in lower parts or strigulose or spreading-pubescent, the upper parts canescent-strigulose, or spreading-pubescent, or with long curly hairs; lower leaves in sort of rosette, the blades rhombic-obovate to rhombic-lanceolate or oblanceolate, acute to obtuse, subentire or remotely denticulate or even pinnatifid, 2–8 cm. long, 0.5–3 cm. wide, narrowed into slightly winged petioles of same or less length; cauline leaves gradually somewhat reduced, becoming sessile or nearly so, and often more deeply sinuate-dentate or pinnatifid, often acute or even acuminate; flowers single in axils, usually in upper half of plant, opening in evening but lasting well into next day; buds drooping, obtuse to acute or acuminate; calyx-tube fairly slender, 2–4 cm. long, slightly widened at summit, strigulose or with spreading hairs (rarely glabrous) without, finely puberulent within tube but quite glabrous toward expanded summit; calyx-lobes greenish to

reddish, but usually hoary with pubescence, distinct and reflexed in anthesis, lance-linear, acuminate, 0.8–3 cm. long and 2.5–4.5 mm. wide, with or usually without free tips; petals white, pink in age, orbicular-obovate to -obcordate, 1–4 cm. long, slightly wider, with or without shallow terminal sinus, not clawed; stamens subequal, filaments flattened, 0.4–0.7 mm. wide, half as long as petals; anthers glabrous, yellowish, 3–9 mm. long; pistil slightly exceeding stamens and not equaling petals; style glabrous; stigma-lobes 3–6 mm. long; capsules spreading, even reflexed, woody, with exfoliating epidermis, prismatic-cylindric or cylindric, usually tapering gradually from base toward apex, dehiscing septicidally, 2–7 cm. long, 1.5–5 mm. thick at base; seeds narrowly obovoid, 1.5–2 mm. long, when thoroughly matured light brown and usually with purple spots and rows of very minute cellular pits.

KEY TO VARIETIES

Hair of calyx-lobes, calyx-tube and upper stems spreading, not at all closely appressed (see also forma *glabrata* under var. *Piperi*).

Petals usually less than 2 cm. long; uppermost leaves deeply sinuate-dentate or pinnatifid, with portion along midrib 3–4 mm. wide; buds with long curly hairs 2 or more mm. long; capsules 1.5–3 cm. long and 3–5 mm. thick at base. Eastern Oregon to Inyo Co., California and adjacent Nevada ..... 1*a*. *Oe. deltoides* var. *Piperi*.

Petals 2–4 cm. long; uppermost leaves usually less deeply divided, rarely pinnatifid; hair on buds various; capsules 2.5–7 cm. long. At lower altitudes.

Hair on buds curly, 2 or more mm. long; buds obtuse; capsules 3–5 mm. thick at base. Central valley of California ..... 1*b*. *Oe. deltoides* var. *cognata*.

Hair on buds straight, 1–1.5 mm. long; buds obtuse to acute; capsules 2–3 mm. thick at base. California deserts and in adjacent Arizona ..... 1*a*. *Oe. deltoides* var. *typica*.

Hair of calyx-lobes, etc. closely appressed, or in var. *arizonica* both appressed and spreading.

Calyx with both appressed and scattered spreading hairs (cf. also f. *floccosa*) and with purple spot at base of each spreading hair; cauline leaves usually regularly and deeply pectinate-pinnatifid; capsules very slender, 1.5–2.5 mm. thick at base. Southeastern Arizona ..... 1*d*. *Oe. deltoides* var. *arizonica*

Calyx with appressed hairs only and without purple spots on buds; leaves subentire to shallowly and coarsely dentate; capsules usually 2.5–5 mm. thick at base.

Calyx-lobes with free tips 1–2 mm. long; buds usually 4-angled toward tip. Southwestern Utah and adjacent Nevada and Arizona ..... 1*e*. *Oe. deltoides* var. *ambigua*.

Calyx-lobes without free tips; buds not at all 4-angled toward tips. Southeastern California and adjacent Arizona and Sonora ..... 1*f*. *Oe. deltoides* var. *cineracea*.

1*a*. **Oenothera deltoides** Torr. & Frem. var. **typica** n. nom.

Oenothera deltoides Torr. & Frem. l.c. *Oe. trichocalyx* f. *albicauloidea* Levl., Mon. Onoth., 346. 1909. *Oe. trichocalyx* f. *mollis* Levl., l.c. *Oe. trichocalyx* f. *refracta* Levl., l.c.

Plants 2–10 dm. high; upper leaves not differing much from others, lanceolate to rhombic, shallowly or coarsely dentate, but not at all pinnatifid; hairs on upper parts of plants short and fairly straight, 1–1.5 mm. long, spreading; buds obtuse to quite acute; petals 2–4 cm. long; capsules 4–5 (7) cm. long, commonly 2–3 mm. thick at base.

Type locality not given, but probably on the Mohave Desert of California. Representative of material studied, CALIFORNIA: San Bernardino County: Barstow, *Minthorn 77* (C, US), *Munz 2556* (P); Camp Cady, *S. B. & W. F. Parish 1306* (G); Ludlow, *Munz & Harwood 3414* (P); Needles, *Jones 3870*, with very short free calyx-tips (C, P, US). Riverside County: Blythe Junction, *Munz & Harwood 3583* (P, US); Indio, *Spencer 1447* (G, P); Palm Springs, *Parish 4107* (C, G, US); Whitewater, *Wright in 1880* (G, S); McCoy Wash, *Hall 5926* (C, US). San Diego County: San Felipe, *McGregor 2047* (S); Borregos Springs, *Brandegee in 1905* (C); Cariso Creek, *Brandegee in 1901* (C). Imperial County: Brawley, *Parish 8326* (G, S); Signal Mt., *Abrams 3192* (G, S). ARIZONA: Ft. Mojave, *Cooper in 1861*, in part (G, US); Mohawk, *Peebles & Harrison 5026* (US). LOWER CALIFORNIA: 6 miles south of San Quentin, *Ballou & Canby 55* (P).

This variety therefore ranges throughout the California deserts, although it is rare on the Mohave Desert, and into adjacent Arizona and Lower California. Specimens from Pinyon Wells in the Little San Bernardino Mts., *Munz 4511* (P) and from Kelso, *Jones in 1906* (P), from altitudes of 4000 and 3000 ft. respectively approach the var. *Piperi* in the longer hair and more finely divided upper leaves.

Although I have not seen the type of *Oenothera deltoides*, I have had the needed information concerning it through the kindness of Dr. Rydberg, describing the type of pubescence and the capsules. Dr. Rydberg writes that the type collection, a Fremont specimen, bears no number nor locality, but that his guess is "that it was collected somewhere between the Mojave Desert and St. George, Utah." This is in agreement with the distribution of plants with this type of pubescence.

1*b*. **Oenothera deltoides** var. **cognata** (Jepson) Munz, n. comb.

Oe. trichocalyx Nutt. var. *cognata* Jepson, Man. Fl. Pls. Calif., 681. 1925.

Plants commonly 2–4 dm. tall and branched from base: basal leaves quite rhombic and glabrate; upper leaves coarsely sinuate-dentate but rarely pinnatifid, with blades commonly 5–10 mm. wide; upper parts of plant well provided with long curly hairs (2 mm. long); buds obtuse; petals 2.5–3.5 cm. long; capsules 2.5–7 cm. long (usually over 3 cm.) and 3–5 mm. thick at base.

Type locality, Corral Hollow, Alameda Co., California.

Ranging from Sacramento south through the San Joaquin Valley of California. Representative of material studied, CALIFORNIA: Sacramento, *Shockley 409* (G); Antioch, *Baker 2873* (G, P, US); Corral Hollow, *Brewer 1217*, type collection (C, G, US); Fresno, *Congdon in 1893* (S); Bakersfield, *Munz 9005* (P); Estrella, *Jared in 1897* (C). Plants from the western end of the Mohave Desert, such as Randsburg-Rand, *K. Brandegee in 1913* (C); Lancaster, *Hall & Chandler 7386* (C), *Elmer 3810* (S), suggest *cognata* and *Piperi* on the one hand by the heavy capsules, but are intermediate with *typica* in the somewhat shorter hair.

1*c*. **Oenothera deltoides** var. **Piperi** Munz, n. var.

Oenothera trichocalyx f. *acaulis* Levl., Mon. Onoth., 347. 1909.

Plants low, frequently less than 1 dm. high (sometimes as much as 3 dm.), often simple; basal leaves rhombic and glabrate; upper leaves lanceolate in outline, deeply and regularly sinuate-dentate to pinnatifid, with rachis 3–4 mm. wide, upper leaves, ovaries and calyces well provided with soft curly hairs ca. 2 mm. long; buds blunt; petals usually less than 2 cm. long; capsules 1.5–3 cm. long, 3–5 mm. thick at base. (Folia superiora profunde sinuato-dentata aut pinnatifida; capsulis mollibus, crispatis, ca. 2 mm. longis; petalis minus 2 cm. longis.)

Type, Man's Lake, eastern Oregon, *W. C. Cusick 2566*, June 13, 1901, Pomona College Herbarium no. 38416; isotypes at University of California, Gray Herbarium, United States National Herbarium.

A plant of the Upper Sonoran Zone ranging from eastern Oregon and northeastern California through western Nevada, at altitudes of from 3000 to 5000 feet. This name is proposed in honor of the late C. V. Piper who had assigned to this concept an herbarium name of specific rank, but the name was never published. Representative of material referred here, OREGON: Steins Mt. *Howell 406* (G); Tule Lake, Klamath Co., *Applegate 897* (US); Lost River, *Austin 1641* (P, US); Pineville, Crook Co., *Leiberg 254* (US). CALIFORNIA: Doyle Station, Lassen Co., *Eggleston 7705* (US); 10 miles south of Amedee, *Jones in 1897* (P); Olanche, *Brandegee in 1891* (C); Constantia, *Kennedy in 1901* (C). NEVADA: Virgin Valley, Humboldt Co., *Kellogg in 1909* (P); Unionville Valley, *Watson 409* (G, US); Washoe Lake, *Jones in 1897* (P, US); Carson City, *Anderson 293* (G); Belleville, Esmeralda Co., *Shockley 224* (C, G, P); Meadow Valley Wash, *Goodding 956* (P).

In northern California there are various intergrades with var. *cognata*, such as *Butler 2000* from Siskiyou Co. (C) with flowers and capsules of *Piperi*, but habit of *cognata*. *M. S. Baker in 1890* from Milford, Lassen Co. (C) has the flowers of *cognata* and foliage of *Piperi*.

In Washoe County, Nevada there occurs a variation with glabrous to glabrate calyx-lobes and tube, but maintaining the other characters of var. *Piperi*, and which may be designated as **Oenothera deltoides** var. **Piperi** forma **glabrata**, new form. I name as type, a collection from Empire

City, *M. E. Jones, June 20, 1882*, Pomona College Herbarium No. 38340. Other specimens belonging here are Pyramid Lake region, *Kennedy 2005*, in part (C, S); Reno, *K. Brandegee in 1883* (C); Washoe Lake, *Baker 1073*, in part (G, P, US).

1*d*. **Oenothera deltoides** var. **arizonica** Munz, n. var.

Simple or branched from base; upper cauline leaves regularly and remotely sinuate-pinnatifid; pubescence of upper parts with very short closely appressed hairs and scattering spreading ones 1.5–2 mm. long; buds acute to obtuse, with purplish spot at base of each long hair; petals 10–25 mm. long; capsules very slender and cylindrical, 35–60 mm. long, 1.5–2.5 mm. thick at base. (Folia superiora certo et remote sinuato-pinnatifida; alabastris strigulosis et pilosis, cum maculis purpureis; petalis 10–25 mm. longis; capsulis tenuibus, 1.5–2.5 mm. crassis.)

Type, Grosetta Ranch near Tucson, Arizona, *J. J. Thornber 509*, April 28, 1903, at 2400 feet altitude, University of California No. 128066; isotype at United States National Herbarium. Material seen, ARIZONA: without locality, *Palmer 596, in 1876* (US), *in 1869* (US); Tucson, *Rose 11878* (US), *Wilcox in 1905* (US), *Peebles, Harrison & Kearney 1273* (US); Gila Bend, *Orcutt in 1902* (C); Phoenix, *Jones in 1903*, in part (P); Casa Grande, *Peebles and Harrison 3536* (US); Lowell, *W. F. Parish 64* (G), *65* (G, S); Florence, *Peebles 1688* (US).

Oenothera deltoides var. **arizonica** forma **floccosa** new form, may be used for Arizona plants which are strigulose in the upper parts, but with the buds having also long hairs so close-set and curled as to appear like tufts of wool. Type, Tempe, *Griffiths 4317*, May, 1903, United States National Herbarium No. 660165. Other collections to be referred here are: Arizona, without locality, *Antisell* (G) · Phoenix, *Jones in 1903*, in part (P); Tempe, *Ganong and Blaschka in 1892* (G).

1*e*. **Oenothera deltoides** var. **ambigua** (Wats.) Munz, n. comb.

Oenothera ambigua Wats., Proc. Am. Acad. 14: 293. 1879. *Anogra simplex* Small, Bull. Torrey Bot. Club 23: 175. 1896. *Oenothera albicaulis* var. *decumbens* Parry? ex Wats., l.c., as synonym. *Oe. trichocalyx* f. *heterophylla* Levl., Mon. Onoth., 346. 1909, in part.

Low stout annuals, 5–25 cm. long; lower leaves rhombic-obovate to -lanceolate, subentire; upper ones more coarsely sinuate-dentate; upper parts with closely appressed hair; calyx-lobes with free tips 1–2 mm. long; petals 13–30 mm. long; buds four-angled toward tip.

Type locality, near St. George, Utah. Ranging in Lower Sonoran Zone as follows, UTAH: southeastern Utah, *Palmer in 1870* (US); St. George, *Palmer 162*, in 1877, type coll. (G, US), *Parry 63* (G). ARIZONA: Littlefield, *Jones in 1926* (P); 8 miles above Pierce's Ferry, *Jones 5077f* (US). NEVADA: Logan, Clark Co., *Heller 10465* (G, S, US); Moapa, *Jones in 1904*

(P); St. Thomas, *Tidestrom 9112* (US); Colorado River, *Goodding 729* (C, G): Valley of Fire, Clark Co., *Jaeger in 1924* (P); 20 miles east of Las Vegas, *Craig 1463* (P).

1*f*. **Oenothera deltoides** var. **cineracea** (Jepson) Munz, n. comb.

Oenothera trichocalyx var. *cineracea* Jeps., Man. Fl. Pls. Calif., 681. 1925.

Habit, foliage, flowers and fruit as in *typica*, but pubescence shorter (less than 1 mm. long) and closely appressed.

Type locality, Borrego Springs, western edge of Colorado Desert, California. Ranging in deserts of Lower Sonoran Zone eastward into Yuma Co., Arizona and adjacent Sonora. Material seen, CALIFORNIA: Borrego Springs, *Brandegee in 1895*, type collection (C); Yaqui Wells, *Jones in 1926* (P); 25 miles east of Holtville, *Munz 7809* (P); sand dunes west of Yuma, *Jones in 1926* (P); Blythe Junction, *Munz & Harwood 3583a* (P); Desert Center, *Jones in 1924*, in part (P); Needles, *Parish 9600* (S). ARIZONA: Mohawk, Yuma County, *Kearney 3930* (US), *Harrison 3580* (US). SONORA: Sierra del Rosario, south of Tinaja Alta, *Lumholtz 13* (G).

2. **Oenothera Engelmanni** (Small) Munz, n. comb.

Anogra pallida var. *Engelmanni* Small, Bull. Torrey Bot. Club **23**: 176. 1896.
A. Engelmanni (Small) Woot. & Standl., Con. U. S. Nat. Herb. **16**: 151. 1913. *Oenothera albicaulis* var. *trichocalyx* Engelm., Amer. Jour. Sci. II, **34**: 335. 1862, not *Oe. trichocalyx* Nutt.

Annual to possibly perennial, erect, 4–6 dm. tall, simple or with few spreading branches, conspicuously throughout except on leaf-surfaces, cauline epidermis exfoliating; leaves quite sessile, lanceolate to oblong-lanceolate, 2–6 cm. long, 1–2 cm. wide, coarsely sinuate-dentate, quite obtuse, canescent, with longer hairs on veins and margins; flowers numerous, buds drooping, shaggy-pilose; calyx-tube slender, 2–3 cm. long, pilose and somewhat strigillose without, finely puberulent within; calyx-lobes 10–15 mm. long, lance-linear, quite without free tips and not adherent in anthesis, both pilose and strigillose; petals 1–1.5 cm. long, broadly obovate, slightly if at all emarginate; filaments slightly flattened, ca. one-half as long as petals; anthers 6–8 mm. long, glabrous; style glabrous, almost equal to petals; stigma-lobes 4–5 mm. long; capsule rather woody, at right angles to stem, somewhat 4-angled, tapering gradually from base, sessile, with exfoliating epidermis, 2.5–4.5 cm. long, 2–3 mm. thick at base; seeds brown, linear-obovoid, 1 mm. long, quite smooth.

Type locality, Las Vegas, New Mexico. Material seen, NEW MEXICO: Nara Visa, Quay Co., *Fisher 54* (US); Roswell, Chaves Co., *Wooton in 1914* (US); on the Cimarron to Santa Fe road, *Wislizenus 473*, given by Engelmann as from Las Vegas, type coll. (G); 5 miles northeast of Portales, *Goodman & Hitchcock 1120* (P). TEXAS: Estelline, Hall Co., *Reverchon 3552* (G, US); Mertzon, Irion Co., *Palmer 12416* (G); south of Mustang Spring, *Havard in 1881* (US); without locality, *Pope* (G).

This species is amply distinct in its sessile leaves, spreading hair, small flowers, lack of free tips to the calyx, linear-obovoid seeds, and spreading capsules.

3. **Oenothera neomexicana** (Small) Munz, n. comb.

Anogra neomexicana Small, Bull. Torrey Bot. Club 23: 176. 1896.

Perennial, from running underground rootstocks, with one or few stems 3–6 dm. tall, fairly slender, erect or ascending, simple or branched from below or above, with exfoliating epidermis, glabrate below, pilose in upper portions, the hairs 1–2 mm. long; leaf-blades oblong to lanceolate or lance-ovate, obtuse to acute, somewhat irregularly sinuate-dentate, pubescent especially below, 2.5–6 cm. long, 6–15 mm. wide, narrowed at base into petioles, these in lower leaves 1–2 cm. long, in upper may almost be lacking; flowers few, buds narrow, acute, conspicuously pilose; calyx-tube slender, 3–5 cm. long, pilose, and strigillose without, finely puberulent within; calyx-lobes linear-lanceolate, 2–3 cm. long, united in anthesis, with free tips ca. 1 mm. long; petals broadly obovate, 2–3 cm. long, with terminal indentation 2–4 mm. deep; filaments flattened, ca. half as long as petals; anthers linear, yellowish, glabrous, 8–12 mm. long; style glabrous, about as long as petals; stigma-lobes 4–5 mm. long; capsule quite cylindric, not woody, 2–3 cm. long, 2–3 mm. thick, sessile, not beaked, straight or nearly so, usually quite erect; seeds dark, obliquely cylindric-ovate, minutely and regularly cellularly roughened under the microscope.

Type locality, "sandy bank of a creek near the Copper Mines, New Mexico." Occurring in the mountains of southwestern New Mexico, the plants from Dona Ana County being somewhat smoother than those to the north and west. Material seen, NEW MEXICO: Wheelers, Socorro Co., *Wooton in 1906* (P, US); Hop Canyon, Magdalena Mts., *Herrick 567* (US); west fork of the Gila, Mogollon Mts., *Wooton in 1900* (US); Mogollon Mts., *Rusby 136* (US); Tierra Blanca Canyon, Sierra Co., *Chapline in 1915* (US); Copper Mines, Grant Co., *Wright 1068*, type coll. (G, US); Pinos Altos Mts., *Greene 194* (G); Organ Mts., Dona Ana Co., *Wooton 663* (US), *Vasey in 1881* (US), *Wooton and Standley in 1906* (US), and *Wooton in 1895* (US).

4. OENOTHERA NUTTALLII Sweet, Hort. Brit., ed. II, 199. 1830

Anogra Nuttallii A. Nelson, Bot. Gaz. 34: 368. 1902. *Oenothera albicaulis* Nutt., in Fraser Cat., n. 54. 1813, name only; Nutt., Gen. N. Am. 1: 245. 1818; not. *Oe. albicaulis* Pursh. *Baumannia Nuttalliana* Spach, Hist. Veg. Phan. 4: 351. 1835. *Anogra Nuttalliana* Spach, Nouv. Ann. Mus. Paris 4: 339. 1835. *Oenothera albicaulis* var. *Nuttallii* Engelm., Amer. Jour. Sci. II, 34: 334. 1862.

Strong perennials with creeping underground rootstocks; stems erect, 4–10 dm. high, often branched above, quite glabrous, with white exfoliating epidermis; leaf-blades pale green, oblong-linear to -lanceolate, usually entire, sometimes remotely denticulate, acute with thick tip, glabrous above, strigose beneath, 2–6 (10) cm. long, 3–5 (10) mm. wide sessile or on

petioles up to 1 or 2 cm. long; flowers with disagreeable odor; buds acuminate; calyx-tube somewhat reddish, narrow, 2–4 cm. long, swollen at base, glandular-pubescent without, puberulent within; calyx-lobes glandular-pubescent, somewhat reddish, lance-linear, 2–3 cm. long, with free tips 1–2 mm. long, all adhering in anthesis; petals obovate, 1.5–2.5 cm. long; filaments flattened, especially toward base, ca. two-thirds the length of the petals; anthers glabrous, 8–10 mm. long; pistil glabrous, exceeding petals; stigma-lobes 5–6 mm. long, greenish; capsule erect, straight, glandular-pubescent, 2–3 cm. long, gradually attenuated toward apex, 2.5–3 mm. thick at base; seeds narrowly obovoid, ca. 2 mm. long, dark, appearing smooth, but minutely and regularly cellular-pitted under microscope.

Type locality, "From the Missourie." Ranging from the Rocky Mountains to Wisconsin and Minnesota. Representative of material studied, ALBERTA: Craigmyle, *Brinkman 643* (P); Rosedale Coulee, *Moodie 1118* (S, US). SASKATCHEWAN: Boulder Lake, *Macoun 72380* (P). MINNESOTA: Muskoda, Clay Co., *Ballard 3084* (G); Mora, Kanabec Co., *Sheldon in 1892* (G). WISCONSIN: Preble, Brown Co., *Schuette in 1900* (C, G, US). NORTH DAKOTA: Leeds, Benson Co., *Lunell in 1896* (G); Tappen, Kidder Co., *Mearns in 1889* (US). SOUTH DAKOTA: Sand Lake, Brown Co., *Williams 215* (US); Minnekakta, Fallriver Co., *Over 16132* (US). NEBRASKA: Chelsea, *Clements 2812* (G, US); Valentine, Cherry Co., *Bates in 1909* (G); Middle Loup River, Hooker Co., *Rydberg 1586* (US); Hot Creek Basin, Sioux Co., *Webber in 1889* (US). MONTANA: Great Falls, Cascade Co., *Williams 152* (US); Bozeman, Gallatin Co., *Blankinship 203* (P, S, US); Livingston, Park Co., *Scheuber in 1902* (US). WYOMING: Pinedale, Sublette Co., *E. B. & L. B. Payson 2808* (US); Centennial, Albany Co., *Nelson 7696* (G, P, US); Encampment, Carbon Co., *Tweedy 4440* (US). COLORADO: Ft. Collins, *Crandall & Cowen 172* (US); Loveland, *Johnston 425A* (US); Denver, *Jones 671* (P, S); 8 miles west of Morrison, *Munz 11, 111* (G); Manitou, *F. E. & E. S. Clements 10* (G, S, US); Oak Creek, Fremont Co., *Brandegee 829* (C).

Through the kindness of Dr. Pennell I have a photograph of the Nuttall collection at the Philadelphia Academy, which agrees in leaf shape, long narrow buds, and other essential features with the concept I am here treating, and which has largely gone under the name *Oe. albicaulis* Nutt. The Philadelphia sheet, does not, however, show the glandular condition which I find so characteristic of this species. Since Nuttall's first use of the name *albicaulis* was as a nomen nudum, and as Pursh used the same name for another species (Fl. Sept. Am. 2: 733. 1814) before Nuttall presented any description (Gen. N. Am. 1: 245. 1818), I am taking up the next oldest name *Oe. Nuttallii* of Sweet.

5. OENOTHERA PALLIDA Lindl., Bot. Reg. 14: 1142. 1828

Perennial, with creeping rootstocks, plant 2–5 dm. tall, usually with main stem erect and several spreading or ascending branches, sometimes

with several stems from base, cauline epidermis white and exfoliating, quite glabrous, or with few scattering long hairs in upper parts, rarely quite cinereous-appressed throughout; cauline leaves mostly lanceolate to lance-linear, subentire to remotely denticulate or sinuate-dentate, usually with margin undulate, acute to acuminate, the blades 2–6 cm. long, 3–10 mm. wide, sessile or short-petioled, usually quite glabrous, rarely cinereous; flowers fragrant; buds acuminate, nodding; calyx-tube very slender, frequently reddish, 2–3.5 cm. long, usually glabrous, occasionally strigulose or with scattering long hairs without, puberulent within; calyx-lobes usually adherent in anthesis, 12–18 mm. long, usually reddish, narrowly lanceolate, with free tips 0.5 to 2 mm. long; petals broadly obovate or retuse, 1.2–3 cm. long; stamens glabrous; filaments slightly flattened, ca. two-thirds the length of the petals; anthers 5–10 mm. long; pistil glabrous, almost equaling petals; stigma-lobes 3–8 mm. long; capsule usually curved, often somewhat glabrate to (rarely) somewhat cinereous, 1.5–4 cm. long, 2–3 mm. thick at base, subcylindric, somewhat but not sharply 4-angled, tapering gradually toward the apex which has 4 short lobes; seeds in 1 row, linear-obovoid, 1.5–2 mm. long, brownish with dark spots, or quite black when ripe; appearing quite smooth, but minutely pitted under microscope.

KEY TO VARIETIES

Glabrous or glabrate..... 5*a*. *Oenothera pallida* var. *typica*.
Cinereous, with closely appressed hairs..... 5*b*. *Oenothera pallida* var. *idahoensis*.

5*a*. **Oenothera pallida** Lindl. var. **typica** n. nom.

Oe. pallida Lindl., l.c. *Anogra pallida* Britton, Mem. Torrey Bot. Club 5: 234. 1894, in part. *Oenothera pallida* var. *leptophylla* T. & G., Fl. N. Am. 1: 495. 1840. *Oe. leptophylla* Nutt., ex Wats., Proc. Am. Acad. 8: 602. 1873. *Anogra leptophylla* Rydb., Bull. Torrey Bot. Club 40: 65. 1913. *Baumannia Douglasiana* Spach, Hist. Veg. Phan. 4: 351. 1835. *Anogra Douglasiana* Spach, Nouv. Ann. Mus. Paris 4: 339. 1835. *Oe. albicaulis* race *transiens* var. *pallida* Levl., Mon. Onoth., 342. 1909.

Plant essentially glabrous or glabrate.

Type locality, "northwest of North America," found by Douglas. Ranging from interior Washington to Idaho, Utah, northern New Mexico and Arizona. Representative of material studied, WASHINGTON: Chelan, Chelan Co., *Jones in 1911* (P); Wenatchee, *Whited in 1896* (US); junction of Crab and Wilson Creeks, Douglas Co., *Sandberg and Leiberg 297* (C, G, P, US); Kiowa, Yakima Co., *Cotton 722* (G, US); Rockland, Klickitat Co., *Suksdorf 970* (C, G, US). OREGON: Biggs, Sherman Co., *Heller 10123* (G, S, US); Riparia, Malheur Co., *Jones in 1905* (P); the Dalles, *Munz 9902* (P). NEVADA: Dry Creek, Austin, Lander Co., *Kennedy 4516* (C, S). IDAHO: Blackfoot, *Jones in 1909* (P); between Twin Falls and Shoshone Falls, *Nelson & Macbride 1336* (G, S), Idaho Falls, Bonneville Co., *Merrill & Wilcox 778* (G, US); Rocky Mts., *Nuttall*, type material of *leptophylla* (G). UTAH: Brigham, Box Elder Co., *Zundel 259* (S); Stansbury Island, Tooele

Co., *Watson 408* (G); Lehi, Utah Co., *Goodding 1165* (G, P, US); Milford, Beaver Co., *Jones in 1880* (P, US); Springdale, Washington Co., *Jones 5249* (C, P, US); Kanab, Kane Co., *Jones 5283* (P, US); Bluff, San Juan Co., *Rydberg & Garrett 9954* (US). ARIZONA: Navajo Well, *Jones in 1890* (P); Cornville, Yavapai Co., *Jones 67* (G); Flagstaff, Coconino Co., *Purpus in 1899* (C). NEW MEXICO: Shiprock, San Juan Co., *Standley 7205* (US); Tunitcha Mts., *Standley 7768* (US); Aztec, *Smith 3933* (P).

Nuttall's *leptophylla* is a narrow-leaved form appearing here and there, but not apparently worthy of recognition nomenclatorially; it is represented by the following collections: Wenatchee, Wash., *Whited in 1896* (US); Washtucna, Wash., *Elmer 1037* (US); King Hill, Idaho, *Nelson & Macbride 1102* (C, G, P, S, US); Boise, Idaho, *Clark 23* (G). In the southern part of its range this species runs into *Oe. runcinata* in the increasingly deep lobing of its leaves: Oak Creek, Ariz., *Rusby 603*, and in many of the sheets cited from southern Utah and northern Arizona and New Mexico. But it seems worth keeping the two series apart, since they represent on the whole two quite different entities. *Oe. pallida* has been confused also with *Oe. Nuttallii*, but is not glandular in the inflorescence, is less tall and more freely branched with the branches wide-spreading, with the leaves more undulate on the margins and the leaves not strigose beneath.

5*b*. **Oenothera pallida** var. **idahoensis** Munz, n. var.

Cinereous with closely appressed hair; the plant compact, intricately branched, 1–2 dm. tall; leaves short, 1.5–2.5 cm. long, oblong-lanceolate; capsules 1–2.5 (3) cm. long. (Planta compacta, cinerea, 1–2 dm. alta; foliis brevibus, 1.5–2.5 cm. longis.)

Type, sand hills, 12 miles west of St. Anthony, Idaho, *Merrill & Wilcox 869*, July 8, 1901, United States National Herbarium 580969; isotype at Gray Herbarium. Additional material, IDAHO: sandhills 14 miles west of St. Anthony, *Merrill 408* (US), *406* (US); St. Anthony, *Merrill & Wilcox 876* (G, US). A collection from Idaho Falls, *Merrill & Wilcox 784* (US), is intermediate between *idahoensis* and *typica* in being quite pubescent.

6. OENOTHERA TRICHOCALYX Nutt., ex T. & G., Fl. N. Am. 1: 494. 1840

Anogra trichocalyx (Nutt.) Small, Bull. Torrey Bot. Club **23**: 174. 1896, in part. *A. rhizomata* A. Nels., Bull. Torrey Bot. Club **26**: 240. 1899. *A. Buffumi* A. Nels., l.c., **27**: 267. 1900. *A. violacea* A. Nels., l.c., **31**: 242. 1904.

Biennial or perennial, with taproot system, the stem simple, or more usually, with several stems from the branching caudex, generally 1.5 to 3 dm. tall, simple or branched above, with exfoliating epidermis and reddish tinge, finely strigulose throughout, and also with scattered long spreading hairs in upper parts; basal leaves subentire to denticulate or even pinnatifid, oblanceolate to spatulate in outline, 1–6 cm. long, 3–15 mm. wide, gradually

narrowed into a winged petiole of about the same length; cauline leaves typically lanceolate to oblong in outline, evenly and sinuately pinnatifid into acute lanceolate lobes, these leaves quite sessile and from about 3 to 6 cm. long, sometimes shorter or less pinnatifid; buds acute; calyx-tube slender, tinged reddish, 2–3 cm. long, pilose and strigulose without, puberulent within; calyx-lobes linear-lanceolate, reddish, usually finely strigulose and with the long spreading hairs quite dense especially in apical half, free tips barely visible; petals yellowish at base, obcordate, 10–18 mm. long, about as wide; stamens glabrous, the filaments slightly flattened and from two-thirds to almost as long as the petals; anthers 4–6 mm. long; pistil glabrous, equaling petals; stigma-lobes ca. 2 mm. long; capsule strigulose and pilose, almost straight and somewhat divaricate to reflexed and even contorted, 1.5–3 cm. long, 1–2 mm. thick at base, subcylindric, very slightly narrowed upward; seeds obovoid, minutely cellular-pitted under a microscope, 1.5–2 mm. long, brown with dark spots to quite black.

Type locality, "Plains of the Platte in the Rocky Mountains." Ranging from Wyoming through western Colorado into Utah. Representative of material seen, WYOMING: Platte Plains, R. Mts., *Nuttall*, photograph of type material at Philadelphia Academy (P); Marquette, northwestern Wyo., *Rose 122* (G, US); Big Piney, Sublette Co., *E. B. & L. B. Payson 4364* (G); Alcova, Natrona Co., *Goodding 168* (C, G, P, S, US); Green River, *Nelson 3064*, type collection of *rhizomata* (G, US); Point of Rocks, *Merrill & Wilcox 596* (G, US), *Nelson 3075*, type coll. *violacea* (G). COLORADO: Grand Junction, *Macbride and Payson 709* (G); Deer Run, *Baker 95* (C, G, P, S, US); Gunnison, *Baker 474* (C, G, P, S, US); McElmo Canyon, *Vreeland 861*, type of *Vreelandii* (New York Bot. Gard.). UTAH: Sunnyside, Carbon Co., *Jones in 1905* (P); Theodore, Uinta Mts., *Jones in 1908* (P); Green River, *Tidestrom 1054* (US); La Sal Mts., Grand Co., *Jones in 1914* (P); Milford, Beaver Co., *Jones in 1880* (P).

A collection from Karshaw, Meadow Valley Wash, in eastern Nevada, *Goodding 956* (C, G, P, US) and another from Tonopah, *Shockley 77* (C, S) seem not quite typical of the species but nearest to it. They are evidently perennial and with proper sort of capsules, and can hardly belong to *Oe. deltoides*. Plants from western Colorado have unusually narrow capsules. On the whole, however, this is a well defined entity and has nothing to do with the more western *deltoides* for which the name *trichocalyx* has generally been used.

7. **Oenothera latifolia** (Rydb.) Munz, n. comb.

Oe. pallida var. *latifolia* Rydb., Contr. U. S. Nat. Herb. 3: 159. 1895. *Anogra pallida* var. *latifolia* Small, Bull. Torrey Bot. Club 23: 175. 1896. *A. latifolia* Rydb., Bull. Torrey Bot. Club 31: 570. 1904. *Oenothera pinnatifida* var. *integrifolia* Gray, Pl. Fendler., 44. 1848. *Anogra cinerea* Rydb., Bull. Torrey Bot. Club 31: 570. 1904.[1]

Perennial from underground running rootstocks; stems 1–several, divaricately branched from base or higher, 1–5 dm. tall; plants strigose-canescent throughout; cauline leaves ovate, oblong to lanceolate, the blades

1–4 cm. long, 4–15 mm. wide, denticulate or more commonly shallowly sinuate-dentate, acute to obtuse, sessile or on petioles 1 cm. or less long; calyx-tube slender, 15–30 mm. long, finely pubescent within; calyx-lobes lance-linear, tinged reddish, 12–25 mm. long, with free tips 1–2 mm. long; petals obcordate, 1.5–2.5 cm. long, about as wide; filaments slightly flattened, half the length or almost equal to petals; anthers 6–9 mm. long; pistil glabrous, equaling petals; stigma-lobes 3–4 mm. long; capsule divaricate to spreading, prismatic-cylindric, 1.5–4 cm. long, almost 2 mm. in diam.; seeds obovoid, 1.5 mm. long, brown with dark spots, fairly smooth.

Type locality not stated in original description, which cites specimens as follows: "Mullen, July 17; Grant County, August 4 (No. 1544). No. 112 of my western Nebraska collection is the same." Then further along appears "It is true that there are some intermediate forms, as for instance No. 1843 of this collection (Mullen, July 17)." I have not seen the July 17 collection from Mullen, but would judge that it should not be taken as the type when the author says that it is intermediate with *pallida* (*Nuttallii* of this paper). I have seen two sheets of *Rydberg 1544*, from Forks of Dismal River, Hooker Co., Nebraska (G, US); while this is from the next county to Grant County and may not be the 1544 referred to in the original description, I hereby designate it as the type collection, since it is representative of the concept that the original author had in mind. Other material which may be taken as illustrative of the species is, SOUTH DAKOTA: Cheyenne River, Fall River Co., *Over 16130* (US); Washington Co., *Over 2418* (US). NEBRASKA: Deuel Co., *Rydberg 112* (US); Grand Island, *Bates 5856* (G); Valentine, *Bates in 1891* (G). WYOMING: Pratt, Laramie Co., *Nelson 8283* (US). KANSAS: Wallace Co., *Hitchcock 159* (G, US); Osborne City, *in 1894* (G). COLORADO: Deertrail, *Johnston 1132* (G); Denver, *Bethel in 1895*, in part (US); Marshall Pass, *Baker 867* (C, G, P, US); Gunnison, *Baker 356* (G, P, US); between Bents Fort and Pueblo, *Fremont 234, in 1845*, type of *cinerea* (New York Bot. Gard.). NEW MEXICO: Pecos, *Standley 4999* (G, US); near Santa Fe?, *Fendler 224* (G). OKLAHOMA: Buffalo, Harper Co., *Stevens 3317* (G). UTAH: Salt Lake City, *Garrett 1815* (G, S); Ft. Douglas, *Jones in 1881* (P); 4 miles east of Salt Lake City, *Munz 11, 115* (C, G, P, US); Ogden, *Ward in 1881* (US).

What I am recognizing as this species proves to be rather a troublesome and variable group. Perhaps in general the plants from South Dakota and Nebraska have the leaves wider and more oblong than do the more western plants from Utah and western Colorado. The method of branching varies from having a central stem with branches at right angles to several stems divaricate from the base. Late in the season, the plants become unusually intricate with short leaves, small flowers, and short capsules, for example: north bench of Salt Lake City, *Garrett 3078* (G); and the Ward specimen already cited from Ogden.

8. **Oenothera runcinata** (Engelm.) Munz, n. comb.

Oe. albicaulis var. *runcinata* Engelm., Am. Jour. Sci. II 34: 334. 1862.

Perennial, from running underground rootstocks, plants 1–3 (5) dm. high, usually freely branched and rather bushy, sometimes glabrous but more commonly hoary-strigulose, particularly in the upper parts, occasionally also pilose; plants quite leafy, leaves not greatly reduced up the stem, blades usually deeply sinuate-dentate, with the teeth usually acute and lanceolate, sometimes broader and even obscure; radical leaves from oblanceolate to lanceolate, the cauline ones lanceolate to lance-oblong in outline, 1–6 cm. long, 4–15 mm. wide; lowermost petioles short and winged, upper leaves sessile; flowers few; buds slender, acuminate; calyx-tube slender, 1.5–3 cm. long, glabrous or more usually hoary without, glabrate within; calyx-lobes lance-linear, somewhat reddish, adherent or not in anthesis, 1–2.5 cm. long, the free tips often very obscure; petals yellowish at base, 10–25 mm. long, somewhat wider, deltoid-obovate, not deeply emarginate if at all; stamens with filaments ca. two-thirds the length of the petals, quite filiform, yellowish at base; anthers 3–6 mm. long, yellow; style glabrous, equaling or exceeding petals; stigma-lobes 3–5 mm. long; capsule terete, spreading or even somewhat deflexed, tapering gradually toward tip, sometimes twisted or coiled, 1–2 mm. thick, 1.5–6 cm. long; seeds obovoid, brown with dark spots, or quite black, ca. 1.5 mm. long.

KEY TO VARIETIES

Plant glabrous; capsules very slender, 4–6 cm. long. Dona Ana Co., New Mexico to Greenlee Co., Arizona and adjacent Chihuahua..... 8b. *Oe. runcinata* var. *brevifolia.*

Plant not glabrous; capsules often shorter and thicker.

Plant hoary, but not conspicuously hirsute.

Leaves deeply sinuate-dentate, green to hoary. Well distributed through Arizona, New Mexico, and western Texas..... 8a. *Oe. runcinata* var. *typica.*

Leaves with shallow teeth or quite entire, hoary. White Sands, New Mexico..... 8c. *Oe. runcinata* var. *gypsophila.*

Plant with long spreading hairs, as well as strigulose, so as to appear quite hirsute; leaf-blades very deeply cut. Socorro Co., New Mexico..... 8d. *Oe. runcinata* var. *leucotricha.*

8a. **Oenothera runcinata** (Engelm.) Munz var. **typica** n. nom.

Oe. albicaulis var. *runcinata* Engelm., l.c. *Anogra pallida* var. *runcinata* Small, Bull. Torrey Bot. Club 23: 175. 1896. *Anogra runcinata* Woot. & Standl., Contr. U. S. Nat. Herb. 16: 151. 1913. *Oenothera albicaulis* race *transiens* var. *tigrina* Levl., Mon. Onoth., 342. 1909, in part.

Plant more or less hoary-strigillose; leaves deeply sinuate-dentate to runcinate-pinnatifid, 2–4 cm. long; capsules ca. 2 mm. thick at base.

Type locality, near Santa Fe, New Mexico. Representative of material seen, ARIZONA: Flagstaff, *MacDougal 116* (C, G, US); Holbrook, *Zuck in 1896* (C, G, US); Moki Reservation, *Hough 29* (US). UTAH: St. George,

Palmer 163 (G). NEW MEXICO: Gallup, *Wooton 2742* (US); Albuquerque, *Greene in 1877* (G); Socorro, *Diehl 597* (P); Grant, Valencia Co., *Munz 11320* (C, G, P, US); Santa Fe, *Fendler 223*, type coll. (G, US); Organ Mts., *Wooton in 1900* (S); Mesilla Park, *Cockerell* (US). TEXAS: Marfa, Presidio Co., *Orcutt 1256* (US); Guadalupe Mts., *Havard in 1881* (US); El Paso, *Jones in 1884* (P); Valley of Rio Grande, 60 miles below El Paso, *Wright 191* (G). CHIHUAHUA: Juarez, *Stearns 331* (US).

This species intergrades with *Oe. pallida* and *Oe. latifolia*. From the former it is separated by its straight spreading capsules more deeply dentate leaves and greater pubescence, as well as somewhat lower stature; from the latter by the narrower leaves which are much more deeply dentate.

8*b*. **Oenothera runcinata** var. **brevifolia** (Engelm.) Munz, n. comb.

Oe. albicaulis var. *brevifolia* Engelm., Amer. Jour. Sci. II, 34: 335. 1862. *Oe. Wislizeni* Levl., Mon. Onoth., 348. 1909.

Plant glabrous; capsule very slender, ca. 1 mm. thick.

Type locality, sand hills south of El Paso. Material seen, ARIZONA: Clifton, Greenlee Co., *Davidson* 240 (S). NEW MEXICO: Las Cruces, Dona Ana Co., *Wooton in 1894* (US); Mesilla Valley, *Wooton in 1893* (US), *in 1907* (US); San Visitas, *Wooton in 1913* (US). TEXAS: El Paso, *Wagner 909* (US), near El Paso, *Wislizenus 99*, type coll. (G). CHIHUAHUA: Paso del Norte, *Pringle 1132* (G). This variety in its glabrous condition resembles the much more northern *Oe. pallida*, but its straighter capsules, more deeply sinuate leaves, and lower stature suggest an affinity with *Oe. runcinata*.

8*c*. **Oenothera runcinata** var. **gypsophila** (Eastwood) Munz, n. comb.

Oe. albicaulis var. *gypsophila* Eastw., Proc. Calif. Acad. III, Bot. 1: 73, pl. 6, fig. 2. 1897. *Anogra gypsophila* Heller, Cat. N. Am. Pls., ed. II, 8. 1900. *Oe. albicaulis* race *transiens* var. *gypsophila* Levl., Mon. Onoth., 342. 1909, in part.

Plant quite hoary; leaves with shallow teeth or subentire.

Type locality, White Sands, New Mexico. Material seen, NEW MEXICO: Otero Co., White Sands, *Wooton 162* (G, P, S, US), *July 19, 1897* (US); Mesilla Valley, Dona Ana Co., *Standley in 1906* (US); White Sands, *Wooton & Standley 3542* (US). It intergrades freely with *typica*, from which it differs only in its greater hoariness and less deeply dentate leaves. Such intergrades are: Mesilla Valley, *Wooton & Standley 3158* (S); Dona Ana, *Wright 1067* (G, US).

8*d*. **Oenothera runcinata** var. **leucotricha** (Woot. & Standl.) Munz, n. comb.

Anogra leucotricha Woot. & Standl., Contr. U. S. Nat. Herb. 16: 151. 1913.

Plant hirsute as well as strigulose; leaf-blades very deeply dentate.

Type locality, San Augustine Plains, Socorro Co., New Mexico. Material seen, NEW MEXICO: San Augustine Plains, *Wooton 2735*, type (US); Willard, *Wooton in 1904* (US).

9. OENOTHERA CALIFORNICA Wats., in Brewer & Watson, Bot. Calif. 1: 223. 1876

Perennial from underground rootstocks, rather coarse-stemmed, rarely simple; stems 1–4 (6) dm. long, frequently decumbent or ascending, glabrous and green or more commonly ashy with short appressed hairs throughout and with some longer ones in upper parts, especially about the flowers, epidermis exfoliating; leaves variable, blades glabrous to hoary canescent and even villous, basal ones oblanceolate to spatulate in outline, cauline oblong to lanceolate, all varying from subentire to deeply and regularly sinuate-dentate, 1–6 cm. long, 0.5–1.5 cm. wide, acute to obtuse, cauline largely sessile or on petioles up to 1 cm. long, lower on petioles 1–3 cm. long; flowers several; buds acute, not very slender; calyx-tube slender, 2–4 cm. long, glabrous to strigulose and villous without, minutely puberulent within; calyx-lobes lance-linear, 1.5–2 cm. long, adherent or quite free in anthesis, with free tips usually obscure, sometimes almost 1 mm. long; petals orbicular-obovate, 2–3 cm. long, even wider, frequently emarginate and with small tooth in the shallow sinus; filaments flattened, ca. half as long as petals; anthers 5–10 mm. long; style glabrous, about equal to stamens; stigma-lobes 4–6 mm. long; capsule terete, usually divaricate, and somewhat curved upwards, 2–5 cm. long, ca. 3 mm. thick at base; seeds plump, obovoid, ca. 1.5 mm. long, brown with dark spots.

KEY TO VARIETIES

Plant more or less gray-pubescent, strigulose and in upper parts also somewhat villous..... 9*a*. *Oe. californica* var. *typica*.
Plant glabrous throughout..... 9*b*. *Oe. californica* var. *glabrata*.

9*a*. **Oenothera californica** Wats. var. **typica** n. nom.

Oe. californica Wats., l.c. *Anogra californica* Small, Bull. Torrey Bot. Club 23: 176. 1896. *Oenothera pallida* var. *californica* Jepson, Man. Fl. Pls. Calif., 681. 1925. *Oe. albicaulis* var. *californica* Wats., Proc. Amer. Acad. 8: 582. 1873. *Oe. albicaulis* race *transiens* var. *melanosperma* Levl., Mon. Onoth., 342. 1909.

Plant strigulose throughout, as well as more or less villous in upper parts.

Type locality, not stated, nor do specimens cited by Wallace, Proc. Am. Acad. 8: 602, seem now available; but if the first collection there listed be taken as type, the one by Wallace, the type locality would be Los Angeles. This variety is a plant common in the interior of central and southern California and ranging into adjacent Nevada and Lower California. Representative of material seen may be cited, CALIFORNIA: Tia Juana River, San Diego Co., *Abrams 3510* (C, G, P, S, US); Jacumba, *Eggleston 19734* (G, P); Warners Ranch, *Keck & McCully 20* (P); Temecula, *Munz 10375*

(P); Santa Ana, Orange Co., *Geis in 1902* (S); Anaheim, *Baker 4117* (P); Hemet Valley, Riverside County, *Munz 5782* (P); Idyllwild, *Spencer 2292* (G); Santa Rosa Mts., *Munz 5845* (P); San Bernardino, *Parish 4152* (C, G, US); Cucamonga, *Abrams 2659* (G, P, S, US); Bear Valley, *Parish 3147* (S); Holcomb Valley, *Munz 10637* (P); 2 miles west of Warrens Well, Little San Bernardino Mts., *Munz & Johnston 5192* (P); Victorville, *Parish 10527* (S); Claremont, Los Angeles Co., *Cowan 34* (P); Swartout Valley, San Gabriel Mts., *Munz 4587* (P); Los Angeles, *Lyon in 1883* (G); San Fernando Valley, *Bush in 1879* (G); Santa Clara River, *Peckham in 1866*, in part (US); Mt. Pinos, Ventura Co., *Dudley & Lamb 4586* (S); Griffins, *Elmer 3591* (G, S, US); Tehachapi, Kern Co., *Eastwood 3257* (G, US); north fork, Kern River, *Palmer 123* (US); Argus Mts., Inyo Co., *Purpus 5416* (G, US); Owens River Valley, *Coville & Funston 1777* (US); 20 miles from Lone Pine, Mono Co., *Ferris 1377* (S). NEVADA: between Austin & Big Creek, Lander Co., *Kennedy 4585* (C, G, S); Austin, *Tidestrom 10968* (US); Tonopah, *Shockley 77* (C, S). LOWER CALIFORNIA: 50 miles southeast of Tecate, *Munz 9550* (P); San Pedro Martir, *Brandegee in 1893* (C).

Oenothera californica, as here recognized, is a very variable thing, running into forms that resemble several of the other species in this subgenus, but on the whole it seems to be a distinct enough entity to merit specific rank. In many ways it is near *Oe. latifolia* of the Rocky Mountains, but with coarser capsules and is more villous, and has the seeds more plump, although plants from the higher altitudes of its range approach *Oe. latifolia;* for example those cited from Mt. Pinos, Griffins, Swartout Valley, Bear Valley, and Holcomb Valley. But in such cases the presence of at least a few long hairs seems to indicate *californica* rather than *latifolia*. It is also near *Oe. pallida*, of which it is made a variety in Jepson's Manual. But even the variety *glabrata*, which of course most resembles *pallida*, and which Jepson refers to *pallida* outright, has wider leaves, coarser capsules, a lower and different habit of growth with different branching (not an erect central stem with divaricate branches, but several more or less decumbent stems), and thicker seeds.

Some of the more notable variations in *Oe. californica* var. *typica* are as follows: (1) shaggy; Indian Wells, Inyo Co., Calif., *Coville & Funston 1012* (US); Pallett Creek, San Gabriel Mts., *Munz 6900* (P); Warrens Well, Little San Bernardino Mts., *Parish 2964* (US) this plant approaching *Oe. runcinata* var. *leucotricha* of New Mexico but with thicker capsules. (2) hoary, as well as shaggy; near Round Valley, Mono Co., *Ferris 1377* (S); Deep Springs Ranch, Inyo Co., *Ferris 6629* (S); Argus Mts., *Purpus 5416* (G, US). (3) leaves almost entire; Mohave River, *Spencer 382* (G); Griffith Park, Los Angeles, *Braunton 448* (US). (4) leaves lanceolate, sinuate-dentate; Argus Mts., *Purpus 5416* (G, US); Austin, Nevada, *A. E. Hitchcock 916* (US).

9*b*. **Oenothera californica** Wats. var. **glabrata** Munz, n. var.

Oenothera pallida of Jepson, Man. Fl. Pla. Calif., 681. 1925, not of Lindley.

Plant glabrous or nearly so throughout. (Planta glabrata.)

Type, Vicinity of San Bernardino, California, at 1000–1500 ft. altitude, June 1, 1896, *Parish 4177* (Gray Herbarium; isotypes at University of California and U. S. National Herbarium). Material seen, CALIFORNIA: without locality, *Vasey 195* (US); Aliso Canyon, Orange Co., *Barber 197a* (C); San Bernardino County, without locality, *Parish 8546* (G, US); Cajon Pass, *Hall 3013* (C), *Johnston in 1920* (P); Mojave River, *Palmer 134* (C); Lytle Creek Wash, *Parish 897* (S, US), *8543* (C, S), *8544* (C, S), *K. Brandegee in 1913* (C); San Bernardino, *Parish 4177* (C, G, US), *in 1900* (S), *11275* (P), *Clark* (P); Colton, *Zumbro in 1905* (P), *Wilder in 1904* (P), *Jones 3189*, in part (US); Riverside, Riverside County, *Hall 4952* (C, S, US); Temecula, *Jones in 1926* (P); Banning, *Spencer 1818* (G).

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CARBOHYDRATE VARIATIONS ACCOMPANYING THE MOSAIC DISEASE OF TOBACCO

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In a previous investigation (Dunlap, 1930) the mosaic disease of tobacco was found to be accompanied by a decrease in the total carbohydrate content of the host plant. To supplement these results, the effects of mosaic upon the amounts of each of the various carbohydrate materials in tobacco leaves were studied. Quantitative analyses were planned for the reducing sugars, non-reducing sugars, dextrin, starch, and pentosans in healthy and mosaic leaves of tobacco, gathered at different times of the day and also in material previously exposed to different conditions of light and of temperature. Variations in the carbohydrate fractions, if found to accompany the diseased condition, might show some disorder to exist in the metabolic activities of the host. By exposing the plants to unusual environmental conditions, any such disorders could be made more apparent by accelerating or inhibiting the activity of the enzyms involved in carbohydrate transformations.

Only a few comparative analyses of the carbohydrates in the tobacco plant under normal and mosaic-diseased conditions have been reported. Bailey (1924) found smaller amounts of sugar and starch in mosaic leaves of tobacco than in healthy leaves. In the case of other virus diseases of certain plants, the effects of the disease upon the sugar and starch contents have also been recorded. Brewer and others (1926) found a reduction of the sugars and polysaccharides in tomato plants affected with the mosaic disease. On the other hand, True and Hawkins (1918), Campbell (1925), and Rosa (1927) have reported increases of carbohydrates in connection with the blight of spinach, potato leaf-roll, and tomato yellows, respectively. Dunlap (1930) found a decrease in the total carbohydrate content of diseased leaves to be characteristic of the several different mosaic diseases studied, and an increase in this content was found to accompany the virus diseases of the yellows type.

MATERIALS AND METHODS

Fully-developed leaves which were in an actively functioning condition and which were secured from tobacco plants of various ages were used in this work. The plants were grown in the greenhouse during the period from October to June. Analyses were made upon three different lots of plants during this time. The mosaic plants were inoculated as seedlings with typical mosaic virus and were kept in the same greenhouse as the

healthy plants. For each analysis a sufficient number of leaves were taken from a few plants to make a composite sample 120 grams in fresh weight. Two samples were taken at the same time, one from healthy plants and one from corresponding mosaic plants. These two samples were treated side by side and in identically the same manner throughout the analytical process. Duplicate samples were taken of plants under each of the four different environmental conditions designated. These analyses were repeated in each of the three lots of plants, thus making a total of 6 analyses which were averaged for the final figures given in the tables.

Immediately after gathering, the leaves were reduced to a watery mass by grinding. The material was then placed in a flask containing sufficient hot 95 percent alcohol to make the final solution between 70 and 80 percent alcohol. A small amount of $CaCO_3$ was added to each flask and the flasks were heated at boiling temperature from 10 to 15 minutes to complete the extraction process. Each sample was filtered through a Buchner funnel and the residue was washed with hot 80 percent alcohol until no chlorophyll appeared in the extract. The filtrate was made up to volume and the residue was dried on a steam sand-bath at 50° C. After drying, the residue was weighed and ground to a fine powder, which was placed in tightly corked vials and later analyzed.

An aliquot representing about one-third of the above filtrate was evaporated to dryness on the sand-bath. All soluble materials in the residue after the evaporation were taken up in about 40 cc. of water. The solution was cleared with neutral lead acetate and deleaded with potassium oxalate. The clearing and deleading were carried out in a centrifuge tube and the precipitates formed at each step were separated from the solution by centrifuging. The cleared solution was decanted and made up to 100 cc. Two 20 cc. aliquots of this solution were taken. To one of these was added enough concentrated HCl to make a 2 percent solution of the acid. This was hydrolyzed for 15 minutes on a boiling water bath, and the solution then cooled and neutralized with NaOH. Both aliquots were made up to 50 cc., the reducing power of each was determined, and the reducing sugars, disaccharides, and total sugars were calculated from these readings.

In the determination of the insoluble carbohydrates, 2-gram samples of the above residue were extracted for 2 hours under a reflux condenser with 100 cc. of 20 percent alcohol. The alcoholic extract was then filtered off, evaporated nearly to dryness, taken up with water, and the reducing power of the solution determined after hydrolysis with HCl and subsequent clearing. The reducing power of this fraction was considered as representing the amount of dextrin present. The residue remaining from the above extraction was dried to remove the last traces of alcohol. It was then placed in 40 cc. of water and boiled for 10 minutes to gelatinize the starch. After being cooled to 40°, a small amount (less than one-tenth of a gram) of

Pangestin[1] was added to transform the starch into soluble materials. The solution was allowed to stand for one hour with occasional stirring. It was then filtered, hydrolyzed, cleared, and its reducing power determined in order to calculate the amount of starch in the sample. The remaining residue was then hydrolyzed in a solution of $2\frac{1}{2}$ percent HCl for 2 hours on a boiling water bath and the pentosan content was calculated from the final reducing power of the cleared, neutralized filtrate.

A very dilute Fehling's solution was used in the above analyses. Solution *A* contained 17 grams of $CuSO_4$ per liter and solution *B* contained 85 grams of Rochelle salt and 24 grams of NaOH per liter. Ten cc. of each of these solutions were placed in a 125 cc. Florence flask and 5, 10, or 15 cc. of the solution to be analyzed were then added. The reducing mixture, made up to 40 cc. in volume, was heated for 5 minutes in a boiling water bath and the cuprous oxid formed was removed by centrifuging. The residual copper was determined by the iodine-thiosulfate titration method, using a solution containing 3 grams of sodium thiosulfate per liter and with soluble starch as an indicator. All of the carbohydrate fractions were determined as dextrose and are expressed as such in the tables, in terms of percentages of the fresh weight of the original sample.

Very small amounts of free pentose sugars were found in the alcoholic extracts of the tobacco leaves by the furfural phloroglucide test and this carbohydrate fraction was not included in the analyses. The reducing sugars probably consisted of dextrose and fructose, although the amounts of these two forms were not separately determined. The most important disaccharide present was apparently sucrose, since there was no increase in the reducing power of the solution containing the alcohol soluble substances after periods of hydrolysis exceeding 15 to 30 minutes. Likewise, glucosides were not considered as being present in appreciable amounts in the tobacco samples, since the regular hydrolysis continued for 4 hours or more did not result in any increase in the reducing power of the total sugar solution. In all cases, periods of hydrolysis exceeding 45 minutes resulted in a loss of reducing power in the solution, due probably to the destruction of a portion of the fructose present. The carbohydrate fraction designated as starch in this work probably includes both the soluble and insoluble forms. The term "pentosans"[2] is intended to represent the hemicelluloses which would be changed to reducing substances in the above procedure.

During the early part of this investigation an attempt was made to determine the amounts of glucose in the alcoholic extracts of the tobacco leaves and to estimate the approximate amounts of fructose by the difference between the glucose and the total reducing sugars. The iodine-thiosulfate titration method was employed and gave good results for the extracts from

[1] A product of the Digestive Ferments Company.

[2] Pentosans probably comprise 90 percent of the furfural-yielding substances in the tobacco plant, the other 10 percent being made up of pectin, uronic acids, and oxycellulose.

healthy plants. With the extracts from diseased plants, however, it was found that some other substance, the nature of which was not determined, was present which reacted like glucose with the iodine solution and thereby gave readings for glucose which were higher than the total amount of reducing sugar in the sample.

Analytical Data

The data representing the more important features of this investigation have been grouped into the first four tables, according to the time of day and to the environmental conditions of the plants at the time of sampling. In these tables the figures representing the maximum and also the minimum content found in any one of the several analyses are given, as well as the average of all the analyses for the particular content and condition concerned. In the last column of each table are figures which show the percentage amounts of each carbohydrate in the mosaic material as related to the corresponding content in healthy plants. The difference between any one of these figures and 100 represents the amount of reduction in that particular carbohydrate fraction, brought about by the disease.

In table 1 are shown the amounts of the various carbohydrates found in

Table 1. *Carbohydrates in Tobacco Plants Sampled in Forenoon*

Type of Carbohydrates	Percentage of Fresh Weight						Ratio Averages Mosaic : Healthy
	Healthy			Mosaic			
	High	Low	Av.	High	Low	Av.	Percent
Reducing sugars	1.06	0.24	0.60	0.46	0.07	0.23	38
Disaccharides	0.34	0.12	0.21	0.21	0.10	0.15	71
Total sugars	1.38	0.36	0.81	0.56	0.18	0.38	47
Dextrin	0.31	0.15	0.25	0.31	0.11	0.19	76
Starch	0.91	0.23	0.67	0.59	0.00	0.27	40
Pentosans	0.42	0.22	0.27	0.26	0.16	0.21	78
Total insoluble carbohydrates	1.66	0.60	1.19	0.95	0.43	0.67	56
Total carbohydrates	2.70	1.09	2.02	1.46	0.71	1.05	52

the tobacco leaves which were gathered early in the forenoon. It may be seen that the mosaic leaves contained much smaller amounts of all the forms of carbohydrates determined, as compared with leaves from healthy plants. The greatest reduction accompanying the diseased condition was found in the reducing sugars and starch, while the contents of dextrin and of pentosans although considerably reduced by the disease were much less affected.

The effects of mosaic upon the various carbohydrates in leaves of tobacco plants collected in the afternoon may be seen in table 2. At this time of day, as in the morning, all the forms of carbohydrates were found to be present in much smaller amounts in the mosaic than in the healthy

plants. In general, the amount of reduction accompanying the diseased condition appears somewhat less in the afternoon than in the morning. Again the greater reduction due to the disease was found in the reducing sugars and in the starch.

TABLE 2. *Carbohydrates in Tobacco Plants Sampled in Afternoon*

Type of Carbohydrates	Percentage of Fresh Weight						Ratio Averages Mosaic : Healthy
	Healthy			Mosaic			
	High	Low	Av.	High	Low	Av.	Percent
Reducing sugars	0.86	0.42	0.69	0.55	0.25	0.36	52
Disaccharides	0.52	0.07	0.29	0.34	0.04	0.21	72
Total sugars	1.23	0.81	1.98	0.65	0.51	0.58	59
Dextrin	0.63	0.19	0.32	0.40	0.16	0.28	87
Starch	1.30	0.48	1.89	0.81	0.25	0.51	57
Pentosans	0.49	0.22	0.33	0.43	0.18	0.27	82
Total insoluble carbohydrates	2.39	0.95	1.57	1.52	0.60	1.06	67
Total carbohydrates	3.45	1.94	2.55	2.07	1.19	1.64	64

In order to determine if extreme environmental conditions, such as darkness or low temperature, might bring about unusual differences between the carbohydrate contents of mosaic and healthy tobacco leaves, certain mosaic and healthy plants were selected and placed in a dark room, and others in a cold room, for from 2 to 3 days before sampling. The temperature of the dark room was approximately the same as that of the greenhouse in which the plants were grown. The cold room, which was also entirely dark, was maintained at a temperature of about 6° to 8° C. The plants in the dark room and those in the cold room were found to have lower

TABLE 3. *Carbohydrates in Tobacco Plants Placed in Dark Room for 2 to 3 Days*

Type of Carbohydrates	Percentage of Fresh Weight						Ratio Averages Mosaic : Healthy
	Healthy			Mosaic			
	High	Low	Av.	High	Low	Av.	Percent
Reducing sugars	0.84	0.29	0.47	0.39	0.05	0.18	38
Disaccharides	0.20	0.06	0.15	0.14	0.03	0.07	47
Total sugars	1.04	0.35	0.62	0.53	0.10	0.26	42
Dextrin	0.26	0.11	0.18	0.16	0.05	0.11	61
Starch	0.54	0.06	0.34	0.16	0.01	0.09	26
Pentosans	0.29	0.20	0.23	0.27	0.20	0.23	100
Total insoluble carbohydrates	1.09	0.38	0.75	0.54	0.26	0.43	57
Total carbohydrates	1.83	0.73	1.37	1.07	0.69	0.69	50

carbohydrate contents in both healthy and diseased conditions than similar plants taken directly from the greenhouse. The figures in table 3 show that the various carbohydrates in the dark-room plants were found to be reduced in amount by the mosiac, with the exception of the pentosan

fraction, which was the same in the mosaic and healthy material. A much greater reduction in the starch content of the mosaic leaves was found in the plants kept in the dark than was previously noted in the plants from the greenhouse. This marked reduction of starch by the disease is shown to an even greater degree in table 4, in plants which were kept in the cold

TABLE 4. *Carbohydrates in Tobacco Plants Placed in Cold Room for 2 to 3 Days*

Type of Carbohydrates	Percentage of Fresh Weight						Ratio Averages Mosaic : Healthy
	Healthy			Mosaic			
	High	Low	Av.	High	Low	Av.	Percent
Reducing sugars	0.83	0.18	0.48	0.51	0.07	0.24	50
Disaccharides	0.51	0.05	0.23	0.25	0.05	0.13	57
Total sugars	1.18	0.28	0.72	0.67	0.12	0.37	51
Dextrin	0.33	0.11	0.22	0.28	0.08	0.17	77
Starch	0.91	0.00	0.34	0.13	0.00	0.04	12
Pentosans	0.42	0.14	0.27	0.29	0.13	0.20	74
Total insoluble carbohydrates	1.59	0.31	0.83	0.58	0.23	0.43	52
Total carbohydrates	2.77	0.63	1.59	1.25	0.35	0.81	63

room. It may also be noted in all of these four tables that the reduction in the total carbohydrates, accompanying the disease, is fairly uniform throughout the different conditions to which the plants were exposed.

Analyses were made of the carbohydrate contents of the dark and of the light areas excised from the same mosaic tobacco leaves. The results of these analyses, given in table 5, show that the reducing sugars, total

TABLE 5. *Carbohydrates of Dark and Light Areas of Mosaic Leaves*

Type of Carbohydrates	Percentage of Fresh Weight						Ratio Light : Dark
	Dark			Light			
	High	Low	Av.	High	Low	Av.	Percent
Reducing sugars	0.62	0.27	0.41	0.52	0.20	0.33	80
Total sugars	0.89	0.68	0.81	1.04	0.44	0.77	95
Insoluble carbohydrates	1.44	0.48	1.01	1.27	0.73	1.04	103
Total carbohydrates	3.15	1.16	2.18	2.82	1.15	1.85	85

sugars, and the total carbohydrates are present in somewhat smaller amounts in the light green than in the dark green areas. The insoluble carbohydrates were slightly more abundant, however, in the lighter areas. By comparing the variations in carbohydrate contents between dark and light areas with the variations between mosaic and healthy leaves, as given in the preceding tables, it may be noticed that the difference is greater between the leaves than between the areas. Consequently it might be inferred that the reduction of carbohydrates in the mosaic leaves is not due to the presence of

the light green areas. It also seems that the darker areas are not normal areas but that all parts of the mottled leaf should be regarded as diseased, at least in regard to the carbohydrate contents.

In table 6 are given the results of analyses of tobacco plants 15 days after thorough inoculation by needle punctures in many places on the stem and leaves. The analyses of check plants are also given. These data show a definite increase in all the various carbohydrates with the exception of the reducing sugars. Ordinary methods of inoculation apparently did not produce any noticeable increase in the carbohydrates of the infected plants. Such an increase of carbohydrate materials may either be due to interference with the translocation process by the sudden introduction of the virus into the stems and petioles, or it may possibly represent merely a temporary stimulation of carbohydrate production. In the latter case the increase might represent only one point in the curve of change in the amounts of carbohydrates following infection. Incidentally an investigation of the carbohydrate contents of mosaic tobacco plants during the complete course of the disease might be of interest in studying its pathological effects.

TABLE 6. *Carbohydrates in Plants 15 Days after Thorough Inoculation*

Type of Carbohydrates	Percentage of Fresh Weight		Ratio Mosaic : Healthy
	Healthy	Mosaic	Percent
Reducing sugars	0.75	0.68	91
Disaccharides	0.21	0.32	152
Total sugars	0.96	1.00	104
Dextrin	0.41	0.55	134
Starch	0.86	1.54	180
Pentosans	0.37	0.59	160
Total insoluble carbohydrates	1.64	2.68	163
Total carbohydrates	3.56	4.68	131

Comparisons between the data presented here and the results of investigations by others are difficult owing to variations in the time periods between inoculating and the taking of samples, to the taking of samples at different times of the day, and to the exposing of the plants to abnormal environmental conditions. The results reported in the literature agree with the results of this work particularly in regard to the reduction in amounts of certain carbohydrate materials in tobacco plants affected with the mosaic disease.

By comparing the data in tables 1 and 2 it may be inferred that the diseased plants may be able, during a period of active photosynthesis, to overcome partially the effects of the diseased condition, at least in regard to the carbohydrate contents. This inference might be made because the mosaic : healthy ratios are considerably higher in the case of the plants which were gathered in the afternoon. In tables 3 and 4, it appears that

the extent to which the carbohydrates in mosaic tobacco plants are utilized, when the plants are exposed to prolonged periods of darkness or low temperature, is comparatively similar to the extent to which they are used by healthy plants under the same conditions. The ratio of the mosaic to healthy plants in regard to the sugar content was, however, higher in the plants kept in the cold room than in those kept in the dark room. On the other hand, the proportion of starch was found to be decreased under these conditions. Also, a greater reduction of the starch content was found in mosaic plants under both the dark room and cold room conditions than was found under the greenhouse conditions. Such results might be interpreted as showing that the translocation process may be interrupted by the mosaic disease, thus resulting in an increase of the soluble sugars. The diastatic activity may be increased through the agency of the virus, with the consequent decrease of the starch content. Most of the data show that the sugars and starch are more readily affected by the mosaic than the other forms of carbohydrates determined and that the dextrin and pentosan contents are less readily reduced to subnormal amounts by this disease.

Summary

1. The amounts of reducing sugars, disaccharides, dextrin, starch, and pentosans in tobacco were found to be reduced by the mosaic disease.

2. The greatest reductions occurred in the sugar and starch contents.

3. Starch was apparently found to be more readily converted into simpler compounds in mosaic plants than in healthy plants.

4. Sugars appeared to accumulate to a greater degree in the mosaic plants.

5. Variations in the carbohydrate contents between light and dark areas of the same mosaic leaf were not as great as the difference between mosaic and healthy leaves.

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MICROSPOROGENESIS IN *BUGINVILLAEA GLABRA*

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INTRODUCTION

Members of the Nyctaginaceae have heretofore received very little attention from a cytological standpoint. The most extensive work of this nature on members of the family is that of Roćen (1927), who has described briefly and figured the development of the microspores and macrospores as well as that of the embryo sac in eight species. His discussion of microsporogenesis includes only two brief references to the genus *Buginvillaea*. He has described macrosporogenesis and the development of the embryo sac in this genus to some extent. Maheshwari (1929) has made an extensive study of the development of the floral parts of *Boerhaavia diffusa*.

MATERIAL AND METHODS

The purpose of the present investigation was to determine the number of chromosomes and to make a study of the meiotic divisions in the microspore mother cells of *Buginvillaea*. Two varieties were growing in the greenhouses at the University of Wisconsin at the time the study was started, namely, *Buginvillaea glabra* Choisy, which has flowers borne on lavender-colored bracts, and the horticultural variety Crimson Lake, which has rose-pink bracts. According to the records available, the plants of *B. glabra* originally came from Bermuda. Specimens were sent to Dr. J. M. Greenman of the Missouri Botanical Garden for identification. The plants of Crimson Lake had been obtained from Florida. Dr. L. H. Bailey considers this variety to be a form of *Buginvillaea spectabilis* Willd. Both these forms bloomed profusely, and material was easily obtained for study. The writer was also able to obtain material of these same forms which had been growing in the greenhouses at Purdue University for many years. The original source of these plants is unknown.

During the winter of 1928–1929, Miss Ruth Lindsay made fixations of material of *B. glabra* in Florida. These plants were growing out of doors. In September, 1928, plants of four varieties of *Buginvillaea* were obtained from the Royal Palm Nursery, Oneco, Florida, and were grown in the greenhouses at the University of Wisconsin. Only one of these, a Crimson Lake plant, has bloomed, and material from it has been obtained for study. It has been considered advisable to limit this report to a study of *B. glabra*, and to use Crimson Lake for comparative purposes only.

At the beginning of the study, Belling's iron-aceto-carmine method was

used to determine the approximate size of buds in which division figures were likely to occur in the microspore mother cells. Buds about 3 mm. in length usually showed some division figures. A representative series of nuclear division stages was obtained by cutting buds ranging from 1 mm. to 10 mm. in length. A number of preparations were obtained in which a single bud showed stages from diakinesis to spore tetrads and others showed stages from synizesis to the homoeotypic division.

The first material was fixed in the summer of 1927, and subsequent fixations have been made from time to time as the plants came into bloom. Since the young flower buds have a tendency to float on the surface of the chrom-osmo-acetic solutions, they were dropped into Carnoy's fluid for a short time (20 to 30 seconds), then transferred directly to either Flemming's medium solution or Taylor's modification of Flemming's. This method, wherein Carnoy's fluid acts as a "sinker," has been used by Kihara (1924) and by others. Such a combination of Carnoy's and Flemming's media has given the most satisfactory results. In a few cases the buds were torn open, care being used not to injure the anthers, and the whole staminal whorl was removed and plunged immediately into the fixing fluid. The material was allowed to remain in the fixing fluid for twenty-four to thirty-six hours, then washed in running water for ten to sixteen hours. After washing, the material was dehydrated, cleared in chloroform, and imbedded in 54° paraffin. Other fixations were made in Carnoy's solution alone, in Benda's and Bouin's fluids, and in Flemming's stronger solution.

After imbedding, sections were cut at thicknesses varying from 5 μ to 25 μ, those between 8 μ and 10 μ being found most useful. The thicker sections were satisfactory for the study of the homoeotypic division and of the development of the microspores. The sections were then mounted, bleached in hydrogen peroxid, stained in Heidenhain's iron-alum haematoxylin or in Flemming's triple stain, and mounted in Canada balsam. Some of the sections which had been stained in haematoxylin were counterstained with fast green. The green stained the microspore mother cell walls, which were not clearly differentiated by the haematoxylin alone.

A particularly useful combination of stains was found to be a 1 percent aqueous solution of methyl green, a 1 percent aqueous solution of acid fuchsin, and a 1 percent alcoholic solution of erythrosin. The sections were stained in the methyl green for one hour, counterstained in acid fuchsin for 1 minute, and then stained in erythrosin for from two to three seconds. With this stain, the chromatic granules and the nucleoli in the early prophases were stained green, and the linin fibers were stained red. In the later stages the chromosomes were stained a brilliant green.

Some of the results obtained from this study have already been reported in a preliminary form (Cooper, 1929).

Observations

Development of the Microspore Mother Cell

Microspore mother cells in the resting stage are polyhedral in shape, and are easily distinguished from the vegetative cells by their dense, granular cytoplasm. The cell walls in the early prophases are very thin. The nuclei, which are almost spherical, range from 7 μ to 8 μ in diameter.

In the nucleus of a young microspore mother cell the chromatic material appears as several well-defined, deeply stained, chromosome-like bodies which are connected by fine threads (Pl. XXII, fig. 1). Among the chromatic aggregations is a large, deeply staining nucleolus.

Not infrequently two of these chromatic bodies are found lying near each other and, because of their similarity in size, they give the impression of a paired arrangement (fig. 2). The lightly stained strands connecting the chromatic bodies appear as very fine threads which stain faintly, and which form an irregular net-like structure. Where two of these threads cross each other, enlarged deeply staining nodes are to be observed. These nodes differ from the previously mentioned chromatic masses by their irregularity of outline. The peripheral aggregations of chromatic material somewhat resemble the prochromosomes found by Rosenberg (1904), Overton (1905), and Darling (1923) in certain dicotyledonous plants, but seem not to be sufficiently numerous to represent the chromosomes.

As the mother cell begins to enlarge, the material of the chromatic bodies seems to flow out along the linin network, forming an irregular reticulum which is made up of single strands (fig. 3). This network is not uniform in thickness, but shows chromatic accumulations at the intersections of the threads. The peripheral chromatic masses become much smaller, but do not disappear (fig. 4).

While these changes in the network are in progress, the large, deeply staining nucleolus apparently is not connected with the reticulum. The cell now grows rapidly and a dense cytoplasmic zone appears about the periphery of the nucleus (fig. 5). This zone has a more or less granular appearance. The cytoplasm near the cell wall contains many conspicuous vacuoles.

Although at this time the nuclear network is composed for the most part of single strands, there is some appearance of a parallel arrangement of the threads. Many nuclei show more paired threads than would be expected from a haphazard arrangement of the chromatic material. As the nucleus continues to grow, some of the cross threads of the reticulum break apart, and the chromatic material collects in masses (fig. 6). These masses (figs. 7 and 8) and the remaining portion of the reticulum draw away from the nuclear membrane in a manner similar to that described by Overton (1909) for *Thalictrum*, *Calycanthus*, and *Richardia* and by Digby (1912) for *Primula*, and synizesis ensues (fig. 9). With the condensation of the

chromatin-linin material, a densely staining knot is formed at one side of the nucleus near the nucleolus (fig. 10). In some instances the nucleolus is more or less surrounded by the condensed reticulum, and can scarcely be distinguished within the knot. However, the nucleus usually lies excentrically in the cell, the chromatic material being always on the side toward the narrower strip of cytoplasm.

During the growth of the microspore mother cells, the cells of the surrounding tapetum have enlarged somewhat and their cytoplasm has become more or less conspicuously vacuolate. While the stages of synizesis are occurring in the sporogenous tissue (fig. 15), the nuclei of the tapetal cells (Pl. XXIII, figs. 16, 17, and 18) divide mitotically as described by Duggar (1899) for *Bignonia venusta*. Maheshwari (1929) also reports that mitosis occurs in the tapetal cells of *Boerhaavia diffusa*. I have been unable to find any evidence of a fragmentation of the tapetal nuclei such as was described by Tischler (1905) in *Ribes* hybrids, and by O'Neal (1920) in *Datura Stramonium*. After this division no cell plate is formed, the tapetal cells remaining binucleate (fig. 19). The daughter nuclei round up and enlarge somewhat and the surrounding cytoplasm becomes more vacuolate (fig. 20). As the tapetal cells continue to develop, these nuclei may move together and ultimately become flattened against each other so as to produce the illusion of a stage in amitosis. At about the time of microspore-formation, the tapetal cells break apart, and ultimately become disorganized.

Preparations which show synizesis are very abundant, and in those which have been heavily stained in haematoxylin and then destained for a long period of time so that the synizetic knot is more or less transparent, it is possible to distinguish the densely staining chromatic bodies (Pl. XXII, fig. 10). These bodies are particularly noticeable in preparations stained with the combination of methyl-green, acid fuchsin, and erythrosin. As the knot becomes looser, loops of threads which appear to be double in some places and single in others are seen to extend outward toward the nuclear membrane (figs. 11 and 12). These threads are now thicker than in the presynizetic stages (fig. 13). The spireme continues to disentangle itself from the knot (Pl. XXII, fig. 14; Pl. XXIII, fig. 21), and thus far no free ends appear except those which are clearly due to cutting. The more deeply staining material is unevenly distributed along the spireme, being somewhat clumped in certain areas (fig. 22).

Figure 23 represents a stage which is probably somewhat analogous to the often-described open spireme. Here the chromatin appears to be evenly distributed along the double spireme. At this stage, a few free ends of the thread can be seen.

Figures 24 and 25 may be interpreted as representing the "second contraction." The massing of the thread around a central point is not as marked as that observed by Allen (1905) in *Lilium canadense*. At this

stage it would appear that the chromatic substance flows along the thread and collects in certain areas. It was pointed out by Overton in 1909 that the "second contraction" phenomenon may occur in plants which possess long chromosomes, but from his studies he concluded that it seems not to occur in plants with short chromosomes. Santos (1924), however, in his study of *Elodea*, a plant with short chromosomes, found a well-defined "second contraction" stage. In my preparations of this stage there is apparently a massing of the chromatic material in certain areas of the open spireme, and a slight contraction. The thinner portions of the spireme are gradually drawn out until they break, and then they are apparently absorbed into the darkly staining bivalent chromosomes. As pointed out by Beal (1928) in *Gossypium*, the chromosomes are thus formed by a transverse segmentation of an already double heterogeneous spireme.

The process of segmentation does not take place simultaneously in all portions of the spireme. Figure 25 shows one segment (*a*) which has been cut off rather early in the "second contraction." After segmentation has been completed, the bivalent segments lie entirely free from one another (fig. 26). The diakinetic chromosomes of Crimson Lake are not so scattered as are those of *B. glabra* at this stage, but remain in a more or less definite order (Pl. XXVI, fig. 51). It is plainly evident in any case that the bivalent chromosomes are not formed by a looping or folding of portions of the spireme, as has been held by Farmer and Moore (1905) and by others who favor the telosynaptic interpretation of meiosis.

In diakinesis ten pairs of chromosomes can easily be distinguished (Pl. XXIII, fig. 28). During the early stages of diakinesis, the individual chromosomes of each pair are somewhat drawn out and there is apparently a splitting of the components of each pair (fig. 27). Thus "four-parted" bivalent chromosomes are formed, similar to those reported by Overton (1909) for several plants. As the chromosomes shorten and thicken, this splitting is almost completely masked. Considerable differences in size are evident between the chromosome pairs. The longer chromosomes usually appear as thick, parallel rods, but in some cases may be arranged so as to form V- or O-shaped figures.

During diakinesis the chromosomes take the stain readily, and the nucleolus seems to be losing its staining properties. Usually one or two pairs of chromosomes are found in close proximity to the nucleolus (fig. 28). In some cases it is clearly evident that one pair is made up of chromosomes which are decidedly different in size (fig. 26, *H*, *h*). In microsporogenesis in Crimson Lake (Pl. XXVI, fig. 50) as well as in one macrospore mother cell of *B. glabra* found in diakinesis (fig. 66), ten pairs of chromosomes can also be counted, one of them showing a difference in size between its univalent components (*H*, *h*).

Toward the end of this period the chromosome pairs tend to condense, and the nucleolus, by now much reduced in size, disappears. At the same

time the nuclear membrane, which has tended to be irregular in outline, becomes no longer distinguishable, and the nuclear cavity shrinks considerably in size.

The cytoplasm has, since just before synizesis, been gradually changing in appearance. The peripheral portion has become more and more vacuolate (Pl. XXII, fig. 14; Pl. XXIII, fig. 21), and the dense cytoplasm near the nucleus has gradually changed from a zone with small and scattered granules to a uniformly dense region of granules. During the stages just following synizesis, fine fibers can be seen in this region, but they are not observed in the later accumulation of granules. This zone seems similar to the "perikaryoplasm" in *Cobaea scandens* described by Lawson (1898) or to the "trophoplasmic granules" found by Mottier (1914) in *Staphylea trifolia*. Definite perinuclear zones have also been described as occurring in *Bignonia* (Duggar, 1899), in cotton (Cannon, 1903*a*; Beal, 1928), in *Calycanthus* (Overton, 1909), in the Cucurbitaceae (Castetter, 1926; Heimlich, 1928), in *Wistaria* (Roscoe, 1927), and in *Thespesia* (Youngman, 1927). The apparent nature of this cytoplasmic zone seems to vary somewhat with the fixative used. In *Buginvillaea* it is most definite in the combination of Carnoy's and Flemming's media. After Carnoy's alone, the zone was not readily differentiated when the preparations were stained in haematoxylin. In preparations of material fixed in Bouin's and Benda's fluids, this region appears as a finely granular substance with definite limits in the cytoplasm.

Devisé (1914), in his study of *Larix*, concluded that the felted perinuclear zone is an artifact due to the action of the fixing fluids. Kirkwood (1907) mentioned a group of rod-like bodies in the cytoplasm surrounding the nucleus of the microspore mother cells of *Micrampelis*. Since he could not establish any relation between these bodies and the nucleolus, he considered that they arise from cytoplasmic fibers.

Heimlich (1928) found a similar zone in *Cucumis sativus* and agreed with Kirkwood that its origin could not be assigned to the nucleolus. In *Buginvillaea* the granules continue to increase in number, but there does not appear to be an increase in the size of the individual granules such as was described by Heimlich. Beal (1928) described a perinuclear zone in cotton which is very similar to that found by the writer in *Buginvillaea* with the exception that in cotton this zone disappears after the heterotypic division, whereas in *Buginvillaea* it (or its substance) recognizably persists throughout both meiotic divisions. It may be found in young tetrads, but disappears shortly after the formation of the microspores.

After the nuclear membrane has disappeared, the chromosomes do not migrate into the perinuclear zone as reported by Denham (1924) for *Gossypium*, but remain within the nuclear cavity. With the disappearance of the nuclear membrane there is a considerable decrease in the size of the nuclear cavity.

Fibers now appear to extend into the nuclear cavity from several points just within, or from the inner surface of, the perinuclear zone (fig. 29). Some of these fibers seem to become attached to the chromosomes. The chromosomes become so arranged as to be massed near the center of the cell. At the same time the peripheral ends of the fibers appear to shift along the inner margin of the granular zone of cytoplasm and unite into several groups to form the multipolar spindle figure (fig. 29), and by a further union of these polar regions the fibers form a bipolar spindle (Pl. XXIV, fig. 30). During the process of spindle-formation the chromosome pairs have become oriented upon it, and the chromosomes of each pair have become closely appressed. The chromosomes are shortly moved into position on the equator of the spindle.

In contrast to the history just outlined, the chromosome pairs of Crimson Lake, in early diakinesis, are formed in such a way that the enlarged areas of the spireme are connected end to end by thin threads. In early diakinesis, these threads can be seen extending between the individual chromosomes. Later, the chromosomes of the pairs round up, yet the pairs retain a somewhat definite arrangement in the nucleus (Pl. XXVI, fig. 51). Toward the end of diakinesis the chromosomes appear to become reattached end to end, thus forming a double chain (figs. 52 and 53). It may be that the chromosomes are actually reunited, or it is possible that the threads connecting the chromosomes have never been completely severed. When the nuclear membrane disappears, the chromosomes of this variety form a clumped mass in the center of the nuclear cavity. The spindle is formed in a manner similar to that described for *B. glabra*, and the chromosome pairs are drawn on to the spindle. During the heterotypic division the chromosomes of Crimson Lake seemingly separate as rounded units. Toward the end of the anaphases the chromosomes appear to unite laterally (figs. 60, 61), so that they form a chain at the time they reach the poles. In the early telophases the chromosomes again appear as separate units (fig. 62). Here again it may be that the attachments formed in late diakinesis have not been actually severed during the equatorial plate stage. This end-to-end union of the chromosome pairs suggests a condition similar to that described by Gates (1908) and others for *Oenothera*, by Stout (1912) for *Carex*, and by Miss West (1930) for *Viola Riviniana*. In different species of *Oenothera*, however, as described especially by Cleland (1922), the *single* chromosomes are attached end to end during the heterotypic division in a continuous ring (or rings), the number in the ring varying with the species, and alternate chromosomes pass to each pole. The situation found by Stout in *Carex* and by Miss West in *Viola* is quite similar to that found in *Buginvillaea*, var. Crimson Lake. In these plants the chromosome *pairs* are attached end to end in late diakinesis, the spireme thus being obviously double. The chromosomes of each pair then become so closely appressed to each other that there appears to be a single row of chromosomes at the time the chromosomes become arranged on the equatorial plate.

On the heterotypic equatorial plate the chromosomes of *B. glabra* are closely grouped, so that it is somewhat difficult to make an accurate count in lateral view. As they separate, the attachment of the spindle fibers is seen to be telomitic. The chromosomes are somewhat rounded, and constant differences in size appear between the respective pairs (Pl. XXIV, fig. 31). Two pairs are very small, four pairs are intermediate in size, and three pairs are relatively large. Among the three large pairs, one is usually conspicuous in that the two homologous chromosomes of this pair adhere to each other as they are pulled to the respective poles, and thus are drawn out into a dumb-bell shape before separating (fig. 35). The final rupture between the chromosomes of this pair may not take place until they have passed fully half way to the poles. The tenth pair consists of a large (H) and a small (*h*) chromosome (figs. 30–36). This heteromorphic pair separates so that the large chromosome passes to one pole, the small one to the other pole.

Because of the aforementioned close grouping of the chromosomes in the equatorial plate, one cannot always distinguish the unequal pair. However, when the chromosomes are somewhat separated on the spindle, and in those cases in which the unequal pair is seen in profile at the margin of the plate, the inequality between its constituent chromosomes is clearly evident (figs. 32, 33, 34, *H*, *h*).

The heterotypic equatorial plate of Crimson Lake also shows an unequal pair of chromosomes (Pl. XXVI, figs. 54–59, *H*, *h*). In this variety all the chromosomes seem to be slightly larger than the corresponding ones of *B. glabra*. The heteromorphic pair of chromosomes is easily distinguished when that pair is seen in profile.

The early anaphases of *B. glabra* show the separation of the heteromorphic pair (fig. 35). When the anaphase chromosomes are seen in polar view, they are similarly arranged in the two groups on the same spindle (fig. 36). In one group the large chromosome (*H*) is found in the same relative position as the small one (*h*) in the other group. There are clearly ten chromosomes in each group (fig. 37). Occasionally a slight lagging of one pair of chromosomes was noted as they moved to the poles. These chromosomes reach the poles before the daughter nuclei are organized, and in no cases have chromosomes been found outside the daughter nuclear membranes.

The chromosomes give the appearance, in some instances, of being split as they reach the poles (figs. 38, 39). They become grouped together and nuclear membranes soon enclose the daughter nuclei. The nuclei now enlarge considerably, and the apparently paired chromatin masses spread out, forming irregularly shaped lumps connected by thin linin threads (fig. 40). A nucleolus appears in each daughter nucleus. No cell plate is formed between the two daughter nuclei. I have not in any case observed thickenings on the spindle fibers, midway between the nuclei, such as are so often described at this stage.

During interkinesis the daughter nuclei are somewhat ovoid in shape, being slightly flattened on the side toward the equatorial region (fig. 40). What appears to be a large barrel-shaped spindle is developed between the daughter nuclei. Closer observation shows, however, that the outer regions of this spindle do not contain spindle fibers, these being limited to the central area.

The perinuclear zone with its innumerable granules persists as a cylindrical sheath about the spindle. As the spindle fibers disappear, the granules located in this zone flow in from all sides and occupy a broad region between the daughter nuclei (fig. 41). More or less similar zones have been seen by Stevens (1905) in *Botrychium virginianum;* by Yamanouchi (1908) in *Nephrodium molle;* by Youngman (1927) in *Thespesia populnea;* by Roscoe (1927) in *Wistaria sinensis;* by Condit (1928) in two species of *Ficus;* and by Hagerup (1928) in *Pyrola minor*, *Phyllodoce coerulea*, and *Arbutus andrachne.*

The condition in *Buginvillaea* closely parallels that found by Roscoe in *Wistaria*. In *Buginvillaea*, however, the dense granular material not only occupies a broad region between the daughter nuclei, but a thin zone of granules extends outward so as partly or entirely to surround each nucleus.

The daughter nuclei now become more nearly spherical in shape. Since early diakinesis, the mother cell wall has gradually thickened and the cell has assumed a somewhat ovoid shape.

The homoeotypic spindle fibers appear in the clear nuclear regions after the membranes of the daughter nuclei have disappeared. The two spindles usually lie at right angles to each other (fig. 41), although in many cases they are almost parallel. With the development of the spindles, the granules in the zone on the outer face of the nucleus disappear.

The chromosomes, which display less marked differences in size than do those on the heterotypic spindle, become arranged on the equator of each spindle (fig. 41). In this division the chromosomes are so closely packed together that it is difficult to make an accurate count, and still more difficult to make a comparison of relative sizes. In cases in which the chromosomes are spread out on the spindle, it can be observed that ten daughter chromosomes go to each pole.

The homoeotypic division seems to proceed in a regular way, and the four granddaughter nuclei are organized near the plasma membrane of the mother cell (fig. 42). As these nuclei take on a rounded form, the homoeotypic spindles persist for a short time. The granular zone is still well defined between the two spindles (fig. 43). Cytoplasmic strands now develop from the individual nuclei into this separating layer in a manner similar to that described by Stevens (1905) in *Botrychium*, whose figure 37 is very similar to my figure 43. The spindles and cytoplasmic strands persist for only a short time, and then break down. No traces of cell plates have been observed on these spindles.

The substance of the granular zone now appears to divide or to be pulled out into four parts, each forming a dense zone over the inner face of a nucleus (fig. 44). The nuclei move somewhat farther apart and, as reported by Castetter (1926) for *Cucurbita maxima*, "the cytoplasm extending between any two of the nuclei now appears quite fibrillar and closely resembles a spindle." This spindle-like appearance, however, is due to the formation of elongated vacuoles in the central region between the nuclei (fig. 44).

A clear region now appears between the cytoplasm and the cell wall midway between each two nuclei, and a membrane delimits the cytoplasm from this hyaline area. This is the first visible evidence of constriction of the mother cell (fig. 42). The cytoplasmic constrictions progress inward along the vacuolated regions between the nuclei (Pl. XXV, figs. 43–45) until finally the furrows meet at the center of the cell and cut the tetrad into four microspores (fig. 46). As observed by Castetter (1925) in *Melilotus*, the furrowing is brought about in part by the fusion of the small vacuoles that are found in a central region between the nuclei. Possibly the plasma membrane is the active agent which brings about the breaking of the cytoplasmic strands between the vacuoles. While the furrows are developing, the cell wall becomes thicker in the regions opposite the furrows, and it appears as though projections of the cell wall have followed the furrow inward (fig. 44). It is probable that the material which makes up these projections may have been deposited by the constricting cytoplasm, as described by Gates (1924) in *Lathraea* and by Shimamura (1929) in *Rumex*. The projections of the mother cell wall are not thin like the callose walls found by Castetter (1925, 1926) in *Melilotus alba* and *Cucurbita maxima*, but are broadly pointed, more like those described by Farr (1916) in *Nicotiana* and by Shimamura (1929) in *Rumex*. My observations agree with those of Bolenbaugh (1928), who found that in *Tropaeolum majus*, during the process of furrowing, the nuclei move away from the plasma membrane, and that, by the time the cytoplasm is cut into four separate masses, a nucleus occupies a central position in each of the developing microspores.

The preparations used for the study of quadripartition were stained in Heidenhain's iron-alum haematoxylin and counterstained in fast green. The green brought out the cell wall clearly. The thickened walls of the mother cell plus the new ingrowths which have united in the center bind the four young spores together until such time as each spore develops a cell wall of its own. With the appearance of the newly developed cell walls of the spores, the thickened mother cell wall disintegrates and the four young microspores are liberated (fig. 47). The wall of each spore is more or less irregular along the basal side.

Each microspore grows rapidly to several times its original size, and the exine and intine are much thickened (fig. 48). The cytoplasm becomes

highly vacuolate, and ultimately the nucleus moves toward one side of the cell and seems to shrink (fig. 49). No nuclear division has been observed to occur in the microspore, and in all the material examined no pollen grains were observed with two nuclei. The contents of the cell, both cytoplasmic and nuclear, condense to form a small mass in contact with the spore wall on one side. The thick exine and intine usually aid in retaining the approximately hemispherical shape of the cell, although many spores show a complete collapse.

Development of the Macrospore Mother Cell

Both meiotic divisions have taken place in microsporogenesis and the tetrad stage is reached by the time the nucleus of the macrospore mother cell reaches the condition of synizesis. In the late synizetic stages the paired threads are evident (Pl. XXVI, figs. 63, 64). At this time there is a rapid growth of the nucellus and the surrounding integuments. Of the many buds which have been sectioned during the course of this study, two show the nucleus of the macrospore mother cell in diakinesis (figs. 65, 66). In both cells ten pairs of chromosomes are easily recognized. In one instance (fig. 66, *H*, *h*) the chromosomes of one pair appear to be unequal. In the other case (fig. 65) the nucleus was cut, and there is some uncertainty as to the identity of the chromosome pairs. In a late prophase stage an unequal chromosome pair is visible (fig. 67, *H*, *h*).

After nuclear division the macrospore mother cell divides, forming an axial row of two cells. Oftentimes after the first division of the mother cell, both daughter cells disintegrate and the nucellus may complete its growth, but is composed of vegetative tissue only. In other cases, the daughter cells of the macrospore mother cell divide, forming a row of four macrospores, all four of which may disintegrate (figs. 68, 69). In the material studied only two macrogametophytes have been observed. One of these is in the binucleate condition and appears normal (fig. 70). The other one has seven nuclei, which vary considerably in size (fig. 71). The body of this macrogametophyte is highly vacuolate. In many cases the ovule has disintegrated before the opening of the flower (fig. 72). There is thus almost if not quite total sterility in the female line.

Chromosome Number

The number of chromosomes found in the two species of *Buginvillaea* studied is $n = 10$, $2n = 20$. Tischler (1908) found that $2n =$ about 16 in two species of *Mirabilis*, and also in a hybrid resulting from a cross between the two species. *Mirabilis* is the only other genus of the Nyctaginaceae in which the chromosome number has been reported. The haploid number of chromosomes in *Thelygonum Cynocrambe* (a member of the Cynocrambaceae closely related to the Nyctaginaceae) has been found by Schneider (1913) to be 10.

Pollen and Fruit Sterility

The pollen produced by the two forms of *Buginvillaea* studied is, under greenhouse conditions, sterile in so far as I have been able to determine. At the time of dispersal, the pollen is somewhat shrunken in appearance and at no stage in its development have I been able to observe either the division of the microspore nucleus or the presence of two nuclei in the pollen grain. Attempts have been made to germinate the pollen grains to determine the percentage, if any, of viable grains. Pollen from more than 200 flowers was placed in sugar solutions of concentrations varying from 2 to 20 percent. The pollen was examined at the end of 24 hours and again at the end of the second day, and no germination had taken place. The introduction of a portion of a stigma into the drop of sugar solution did not stimulate germination.

The apparent inability of the pollen to germinate and the total or nearly total sterility of the macrogametophyte seem to explain the fact that the forms in question rarely, if ever, produce seeds. I have conducted a series of pollination experiments in an attempt to secure seeds. Pollen of *B. glabra* has been placed on the stigmas of freshly opened flowers of Crimson Lake, and similarly the pollen of Crimson Lake was transferred to the stigmas of the flowers of *B. glabra*. The pollinated flowers were then tagged, and following fifty such cross-pollinations no seeds or fruits were formed. In both forms pollen was also transferred from the anthers to the stigma of the same flower, and from the anther of one flower to the stigma of another flower on the same plant. No results were obtained from the 200 attempted pollinations of these types.

Since seeds were not produced under greenhouse conditions, efforts were made to obtain seeds from tropical regions where these species are native, or have been introduced. In reply to requests for information concerning the fruiting of *Buginvillaea*, information was obtained from a correspondent in Jamaica that seeds have been obtained from a cross between a red variety and a purple one. According to Dr. M. S. Goodman, Superintendent of Hope Gardens, Jamaica, seeds are not produced in Jamaica. He added, however, that he understood that the plants set seed in Trinidad. Both Dr. R. O. Williams, Assistant Botanist, St. Clair Experiment Station, Trinidad, and Dr. E. E. Cheesman, Professor of Botany, Imperial College of Tropical Agriculture, Trinidad, reported that these plants do not set seed with them. Dr. Cheesman reported, however, that a cross between two species of *Buginvillaea* has been made in St. Vincent, B.W.I. A similar report came from a correspondent in Jamaica. Neither of these reports could be verified by the writer.

Both Dr. A. A. Hunter, Botanist at the Tropical Station of the Missouri Botanical Garden at Balboa, C.Z., and Dr. J. E. Higgins, Agronomist in charge of the Plant Introduction Gardens at Balboa Heights, C.Z., have

done considerable work with *Buginvillaea*. According to information from these gentlemen, neither has seen any seeds on the varieties growing in the Canal Zone. Similar reports have come from various parts of South America, as well as from Florida and California.

Discussion

The presence of a heteromorphic pair of chromosomes is, to say the least, an extremely rare occurrence in plants except in certain dioecious species in which the inequality of the chromosomes could or might be associated with sexual differentiation, or in hybrids where, if the chromosomes from the two parents pair, unequal dyads are now and then to be expected.

The number of dioecious angiosperms wherein nequal pairs of chromosomes are reported in the microspore mother cells steadily increasing. Two cases of heteromorphic pairs of chromosomes have been reported in non-dioecious (hermaphroditic) plants. Bělař (1925) found, in a diploid hermaphroditic plant of *Melandrium album* (*Lychnis dioica*), that both pollen mother cells and embryo-sac mother cells possess an $X-Y$ chromosome pair in addition to the regular complements of chromosomes. His conclusion is that this hermaphroditic plant was a modified male. The staminate plants of this species are well known to possess an $X-Y$ pair. Comparable is Hagerup's (1927) finding of 2 X's and 2 Y's in an hermaphroditic tetraploid form of *Empetrum nigrum* whose diploid form is dioecious. Although the condition found in these forms is suggestive of that found in *Buginvillaea*, it must be remembered that they are forms clearly derived from dioecious species with heterochromosomes, whereas there is no reason to assume such an origin for the forms of *Buginvillaea* studied.

It is not impossible that these cultivated forms of *Buginvillaea* are derived from natural hybrids, and that one of the chromosomes of one parent varied in size from the complementary chromosome from the other parent; but evidence of the hybrid origin of either form is quite lacking. Cases have been reported of the origin of hybrids in consequence of crossings between parents of unequal chromosome numbers and size. The classical case of this type is that in *Drosera* (Rosenberg, 1909), wherein a sterile hybrid with 30 chromosomes resulted from a cross between *D. longifolia* with 40 small chromosomes ($2n$) and *D. rotundifolia* with 20 large chromosomes ($2n$). In the hybrid ten of the small *longifolia* chromosomes paired with the larger *rotundifolia* chromosomes, and the remaining ten *longifolia* chromosomes remained unpaired.

Dr. Barbara McClintock, in a personal letter to the writer, says that she has produced crosses between strains of *Zea Mays* in which certain chromosomes of the parents can be identified. In certain strains the second smallest chromosome possesses a terminal enlarged accumulation of stain-

able material; other strains have no such accumulation. Crosses between strains with, and those without, this accumulation give F_1 progeny which are heterozygous for this character; *i.e.*, one chromosome of the pair possesses the mass of stainable material and the other chromosome does not. This statement holds for both reciprocal crosses. Dr. McClintock has further found that there are such accumulations on other chromosomes, mostly a short distance in from the end of the long arm of the chromosome. These show a similar behavior.

The possibility that the two species of *Buginvillaea* studied are hybrids is lessened by the fact that both possess a heteromorphic chromosome pair. On the other hand, this conception is strengthened by the occurrence of pollen sterility and by the lack of complete development of the ovule. In some instances in other plants, pollen sterility follows meioses that are apparently typical. Blackburn and Harrison (1924) in a hybrid willow, and Roscoe (1927) in three species of *Wistaria*, report that the tetrads of spores are apparently normal in their early stages, yet delayed degeneration takes place. Because of the production of sterile pollen, Roscoe considered these species of *Wistaria* to be hybrids.

Repeated discussions have appeared of the supposed relationship between sterility and hybridity. Winge (1917) and Longley (1924) found that pollen sterility is closely correlated with a hybrid nature. Jeffrey (1914) and a number of his students (Forsaith, 1916; Cole, 1917; Hoar, 1927; and Roscoe, 1927) have held that when plants, growing under normal environmental conditions, produce a high percentage of sterile pollen, the explanation is to be found in a previous crossing. In contrast, a number of workers (Cannon, 1903*b*; Tischler, 1908; Digby, 1912; Blackburn and Harrison, 1924; Crane and Darlington, 1927; and others) have described known hybrids wherein normal meiosis took place and the pollen was viable. Huskins (1929) objects to the use of meiotic abnormalities and pollen sterility as criteria for hybridity.

Since the material of *Buginvillaea glabra* obtained in Florida showed a condition similar to that grown under greenhouse conditions, and since all reports from the places where this plant is cultivated indicate that this species is sterile, it seems highly improbable that pollen abortion is here due to environmental conditions, although the work of Sakamura and Stow (1926) indicates that climatic conditions may be a causal factor in pollen sterility.

A condition, perhaps suggestive of that found in *Buginvillaea*, was reported by Cardiff (1906) in *Salomonia biflora* (*Polygonatum biflora*), wherein he found that one of the chromosomes appeared to pass undivided to one pole. He was at a loss to explain the phenomenon since this is a hermaphroditic species. The findings of Cardiff, however, have not been confirmed, and in the light of the work of Heimlich (1928) on the cucumber, it is possible that a disappearing nucleolus was confused with a chromosome.

The heteromorphic chromosome pairs, distinct from the sex chromosomes, which have been noted in the primary spermatocytes of certain insects are perhaps more nearly comparable to those found in *Buginvillaea*. Baumgartner (1911) first described an unequal pair in one of the Gryllidae. Payne (1912), Hartman (1913), Voinov (1914), Robertson (1915), Wenrich (1916), and McClung (1928) have also been able to distinguish such an unequal pair in various orthopterans. In a series of studies Miss Carothers (1913, 1917, 1921) found heteromorphic pairs, distinct from the sex chromosomes, in both male and female individuals of several orthopterans. In some cases she found unequal homologues; in others the chromosomes were distinguished by the manner of spindle-fiber attachment.

The origin of these unequal chromosome pairs in insects has been explained in various ways. Payne (1912) suggested that the X-chromosome was possibly at one time a portion of the larger dyad of the unequal pair and had arisen by fragmentation of this large dyad. Robertson (1915) and Wenrich (1916) have both advanced the idea that the inequality is probably due to a reduction by fragmentation or progressive loss of substance on the part of the smaller member of the pair. Robertson (1915) suggested that in *Acridium* the larger dyad consists of the intact dyad and a portion of the fragmented dyad. This conclusion was suggested by the fact that the larger dyad shows a definite constriction. Miss Carothers (1917) has given evidence that this mode of origin may explain the condition in *Trimopteris*, the fragmentation probably having occurred at the point of spindle-fiber attachment. Both Miss Harmon (1920) and Miss Carothers (1921) have offered hybridity as an explanation for the appearance of heteromorphic chromosome pairs.

Three possible explanations may, then, be suggested for the occurrence of the heteromorphic pair of chromosomes in the species of *Buginvillaea* studied. First, it is possible that, during meiosis in an ancestor of these cultivated forms, one chromosome of the pair became fragmented and that, after the union of gametes, only a fragment acted in lieu of the complete chromosome. When the chromosomes pair in preparation for meiosis, this fragment mates with the other complete chromosome of the pair. Since only vegetative reproduction seems to occur in these forms, such a heteromorphic pair might persist indefinitely.

The slight constriction that is usually found in the large chromosome of the heteromorphic pair in Crimson Lake suggests the second explanation. The fragmented chromosome mentioned above may have remained attached to its synaptic mate and both have been drawn to one pole. The complement of chromosomes containing this large chromosome then united with a typical group of chromosomes in syngamy and in the later reduction divisions this large chromosome mated with a normal chromosome.

Third, it is possible that the unequal pair arose as the result of a cross between two fertile species.

Only very tentative conjectures may be offered upon this question until a careful study has been made of those native species, if any, which produce fertile flowers, and of the environmental conditions under which such flowers are produced. In the light of such a study, a satisfactory explanation might be possible of the heteromorphic pair of chromosomes, as well as of pollen sterility.

Summary

1. The results reported in this paper are based on a study of two forms of *Buginvillaea:* namely, *Buginvillaea glabra* Choisy and the horticultural variety Crimson Lake. The latter seems to be a form of *B. spectabilis* Willd.

2. In the nucleus of a young pollen mother cell, accumulations of chromatin, connected by thin linin threads, are found. These are somewhat suggestive in appearance of prochromosomes.

3. As the pollen mother cell enlarges, the chromatic material spreads out along the linin and an irregular network is apparent.

4. Just prior to synizesis, chromatin masses again appear. They now often lie in pairs.

5. The synizetic knot is made up of the chromatic masses and the linin threads. During synizesis a dense "perinuclear zone" appears in the cytoplasm just outside the nucleus. The material occupying this zone becomes very granular and persists, although with changes in distribution, through both meiotic divisions.

6. When the spireme emerges from synizesis, it is continuous and clearly double. The thread consists of thick and thin areas.

7. In the "open spireme" stage the threads are double, and are approximately uniform in size throughout the length of the spireme.

8. During the "second contraction" stage, the spireme shortens and thickens in preparation for transverse segmentation. At this stage it shows a lumping of the chromatic material to form thick and thin portions.

9. The thin portions continue to become thinner until they break. The thicker portions of the spireme now become recognizable as bivalent chromosomes. Early in diakinesis the chromosomes of each pair seem to split, forming tetrads. In some instances one tetrad is seen to be composed of unequal dyads.

10. The spindle fibers appear to originate from the inner surface of the perinuclear zone.

11. The ten bivalent chromosomes now become oriented upon the heterotypic spindle. There are four pairs of small chromosomes, two pairs intermediate in size, and three pairs of large chromosomes. The dyads of one of these large pairs seem to adhere to each other until they are pulled out into a dumb-bell shape, and then they separate. The tenth pair consists of one large and one small chromosome.

12. In late anaphase, the chromosomes show a longitudinal split.

13. During interkinesis the granular material previously occupying the perinuclear zone forms a layer about the spindle. As the spindle fibers break down, the granular material flows in from all sides, forming a densely granular region between the daughter nuclei.

14. No cell plates are formed after either of the meiotic divisions. Two prominent and four less prominent spindles connect the four nuclei after the homoeotypic division. After a time these spindles disappear.

15. Cytokinesis is brought about by a furrowing of the plasma membrane of the mother cell and results in the formation of four microspores.

16. Each microspore increases greatly in size and then forms a thick intine and exine. Its cytoplasm becomes highly vacuolate, and ultimately the nucleus and cytoplasm shrink into a single mass which lies toward one side of the cell cavity.

17. No case was observed in which the microspore nucleus divided.

18. All attempts to germinate the pollen grains proved futile.

19. Attempts were made to bring about the production of fruit and seeds by crushing the mature anther sacs and placing the pollen on the stigma. Both self- and close-pollinations in each form as well as cross-pollinations were made, but in no case did fruits or seeds develop.

20. The development of the macrospore mother cell was followed as far as the material would permit. The reduction divisions may take place, but the macrospores usually disintegrate early and a nucellus containing vegetative tissue only is developed. Only two macrogametophytes were observed, one in the two-nucleate stage, the other showing seven nuclei.

21. According to all information that could be obtained from those places where the plants grow in the open, the cultivated varieties of *Buginvillaea* do not produce seeds. Some information was obtained to the effect that seeds have been produced as a result of a crossing between *B. spectabilis* var. *laterita* and the variety Lady Seaton James. Seeds could not be obtained from any source.

The writer wishes to acknowledge his indebtedness to Dr. C. E. Allen for suggesting the subject of this investigation and thanks are here tendered him for his many helpful suggestions and kindly criticisms. The opinions of the several others who examined a number of the preparations are likewise greatly appreciated.

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EXPLANATION OF PLATES

Most of the figures were drawn with a Zeiss compensating ocular no. 18 and a 2 mm. apochromatic objective N.A. 1.40, at table level with the aid of an Abbé camera lucida. With a tube length of 200 mm. a magnification of about 4500× was obtained. The drawings of the tapetal cells were drawn with the same objective, but the Zeiss compensating ocular no. 12 was used, and a magnification of approximately 3100× was obtained. Since different optical combinations were used in drawings of the cross sections of the anther and in those of macrosporogenesis, the magnification is given for each of those figures. In reproduction the drawings have been reduced one-half.

PLATE XXII

Buginvillaea glabra

FIGS. 1, 2. Nuclei of young pollen mother cells, showing the accumulation of the chromatic material into bodies somewhat similar to prochromosomes.

FIG. 3. Slightly older nucleus. The chromatic material appears to be spread out along the linin network.

FIG. 4. Still older nucleus. The chromatic material forms an irregular network throughout the nucleus.

FIG. 5. Pollen mother cell with a nucleus at about the same stage as in figure 4. The cytoplasm is vacuolate near the periphery of the cell, and somewhat denser near the nucleus.

FIGS. 6, 7, 8. The chromatic aggregations are again apparent in the nucleus. Figures 7 and 8 show a collapse of the network.

FIG. 9. Early synizesis.

FIG. 10. Synizesis, showing the chromatic aggregations in the knot.

FIGS. 11, 12. Recovery from synizesis. The nucleus has increased considerably in size. The spireme thread appears to be double as it emerges from the synizetic knot.

FIG. 13. Double spireme emerging from synizesis. The paired chromatic lumps are clearly evident.

FIG. 14. Spore mother cell in which the perinuclear zone has become more definite. The chromatic material has spread out along the spireme threads.

FIG. 15. Cross section of a portion of the anther showing the nuclei of the sporogenous cells in late synizesis. Nuclear division is taking place in some of the tapetal cells. ×1150.

PLATE XXIII

FIG. 16. Tapetal cell. Chromosomes at metaphase.

FIG. 17. Tapetal cell. Lateral view of anaphase.

FIGS. 18, 19. Tapetal cells. Lateral views of telophases.

FIG. 20. Binucleate tapetal cell.

FIG. 21. Spore mother cells at about the same stage as in figure 14.

FIG. 22. Nucleus of pollen mother cell. The double spireme thread is spread out through the nucleus.

FIG. 23. Open spireme stage. The paired threads are approximately similar in thickness along their entire length.

FIG. 24. "Second contraction" stage. The chromatic material is collected in definite areas along the spireme. One pair of chromosomes (*a*) has already been cut off from the spireme.

FIG. 25. The nucleus of a pollen mother cell, showing the formation of chromosomes by segmentation.

FIG. 26. Diakinesis. Ten pairs of chromosomes are evident. Those of one pair (*H*, *h*) are apparently different in size.

FIG. 27. Diakinetic chromosomes, each chromosome showing a second longitudinal split.

FIG. 28. Pollen mother cell at diakinesis. The perinuclear zone is definitely formed, and the cell wall is comparatively thick.

FIG. 29. Multipolar spindle. The nuclear membrane has disappeared.

PLATE XXIV

FIG. 30. Heterotypic metaphase. The granular substance of the perinuclear zone forms a sheath around the spindle. Heteromorphic chromosome pair (*H*, *h*) at the left side of spindle.

FIG. 31. The chromosomes of figure 30.

FIGS. 32, 33, 34. Heterotypic equatorial plates. In each plate the heteromorphic pair (*H*, *h*) is present.

FIG. 35. Early heterotypic anaphase. Not all the chromosomes are shown. The heteromorphic pair (*H*, *h*) is at the right of the figure.

FIG. 36. The two chromosome groups seen in a polar view of a heterotypic anaphase.

FIG. 37. Late anaphase. The substance of the granular zone is beginning to flow in between the daughter nuclei.

FIGS. 38, 39. Groups of late anaphase chromosomes. Each of the ten chromosomes has become split.

FIG. 40. Interkinesis. The granular sheath closely surrounds the remains of the heterotypic spindle, although somewhat constricted in the equatorial region.

FIG. 41. Cell with homoeotypic equatorial plates. The spindles lie at right angles to each other. The granular substance now forms a dense region between the two spindles.

FIG. 42. Four-nucleate stage. Spindles and granular substance are shown.

PLATE XXV

FIG. 43. Spore mother cell showing the disappearance of the tetrad spindles and the beginning of cytokinesis by furrowing. Note the vacuoles appearing in the region midway between the nuclei. The dense granular material is still present between the two spindles.

FIG. 44. Advanced stage in cytokinesis. The granular zone has so divided that a dense region appears inside each nucleus. Note the peripheral position of the nuclei and the vacuoles midway between the nuclei.

FIG. 45. Quadripartition nearly completed.

FIG. 46. A tetrad of newly formed microspores. The mother cell wall is still intact.

FIG. 47. Young microspore, showing formation of exine and intine. The cytoplasm contains many minute vacuoles.

FIG. 48. Microspore showing great increase in size. The nucleus likewise has grown. The cytoplasm contains large vacuoles.

FIG. 49. Microspore with thick exine and intine. The cytoplasm has collapsed to one side of the microspore, carrying the nucleus with it.

PLATE XXVI

Variety Crimson Lake

FIG. 50. Nucleus of pollen mother cell in diakinesis, showing ten pairs of chromosomes, including one heteromorphic pair (*H*, *h*).

FIG. 51. Late diakinesis. The chromosome pairs show definite connections.

FIGS. 52, 53. Multipolar spindles. Chromosome pairs attached end to end.

FIGS. 54–59. Heterotypic metaphase groups. Each group shows the heteromorphic pair (*H*, *h*). Figure 59 is a polar view of a metaphase group.

FIGS. 60, 61, 62. Anaphases. Figures 60 and 61 show the chromosomes attached laterally. In figure 61 the longitudinal split is visible in each chromosome.

Macrosporogenesis in *Buginvillaea glabra*

FIG. 63. Macrospore mother cell, nucleus showing recovery from synizesis. × 3100.

FIG. 64. Double spireme emerging from synizesis. × 3100.

FIGS. 65, 66. Diakinesis. The chromosomes of one pair (*H*, *h*) shown in figure 66 are unequal in size. × 3100.

FIG. 67. A late prophase. The nuclear membrane has broken down. × 3100.

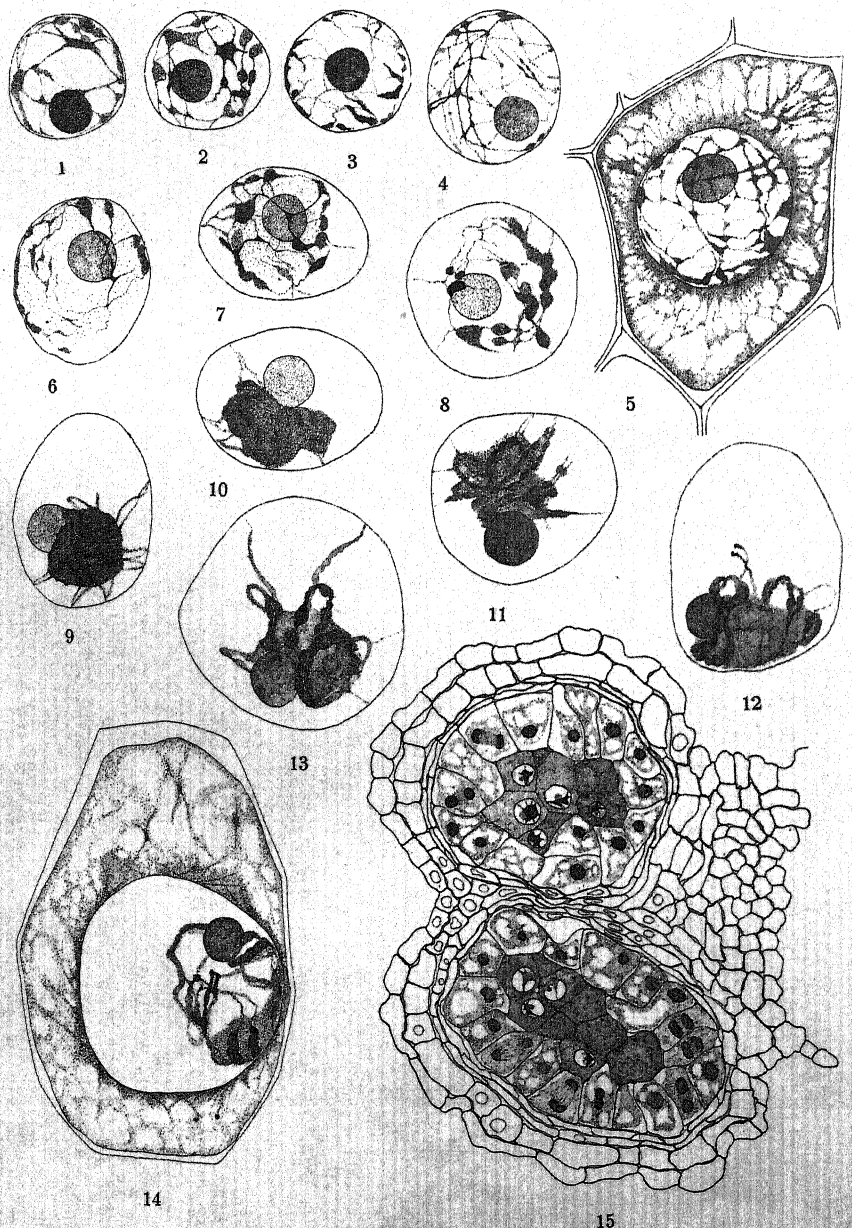
FIG. 68. Three axial cells. The two outer cells are probably formed by the second (homoeotypic) division. The other daughter cell of the heterotypic division has not yet divided. × 1150.

FIG. 69. A stage in the disintegration of the macrospores. The three outer macrospores are collapsed. × 1150.

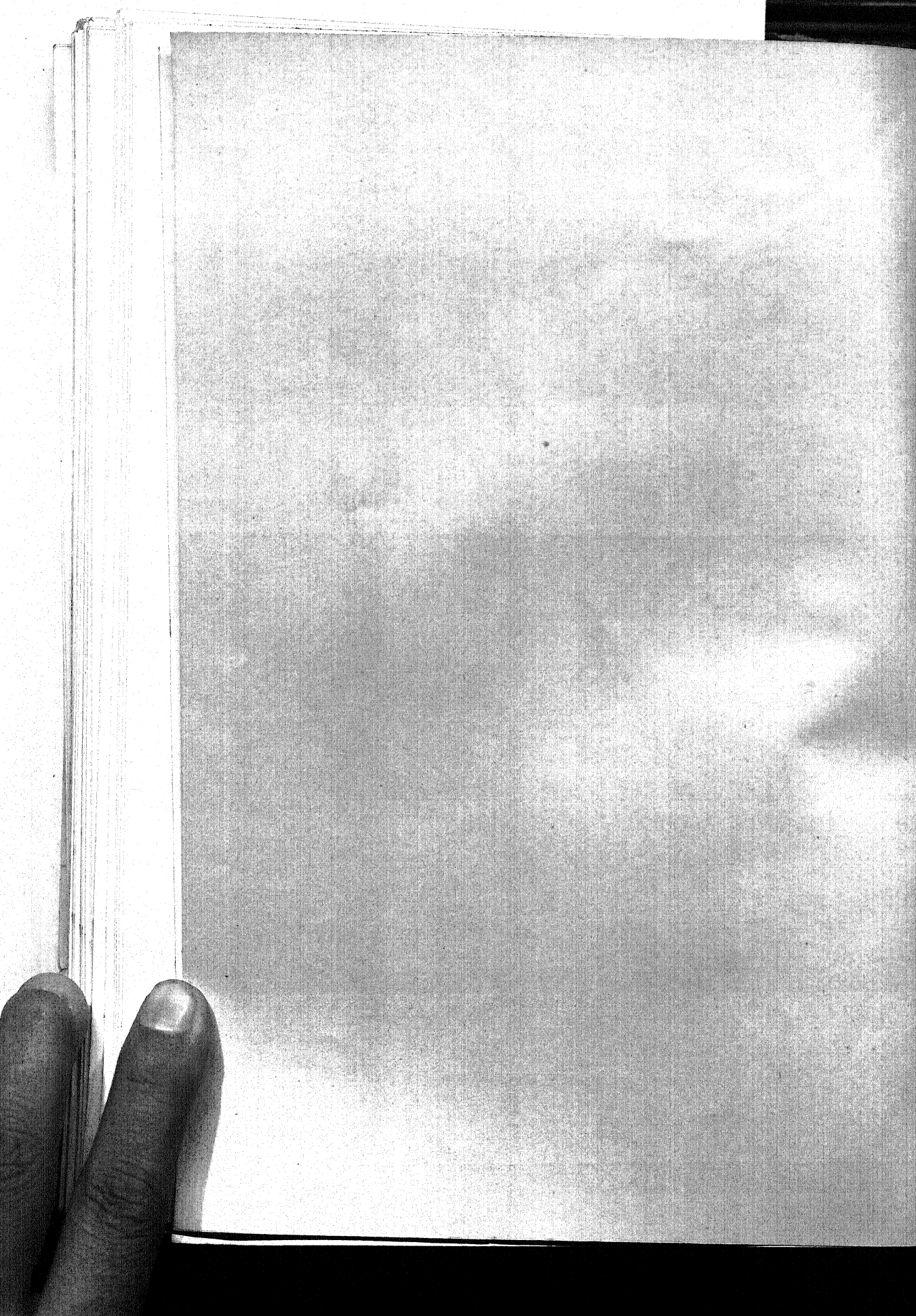
FIG. 70. Two-nucleate macrogametophyte. × 1560.

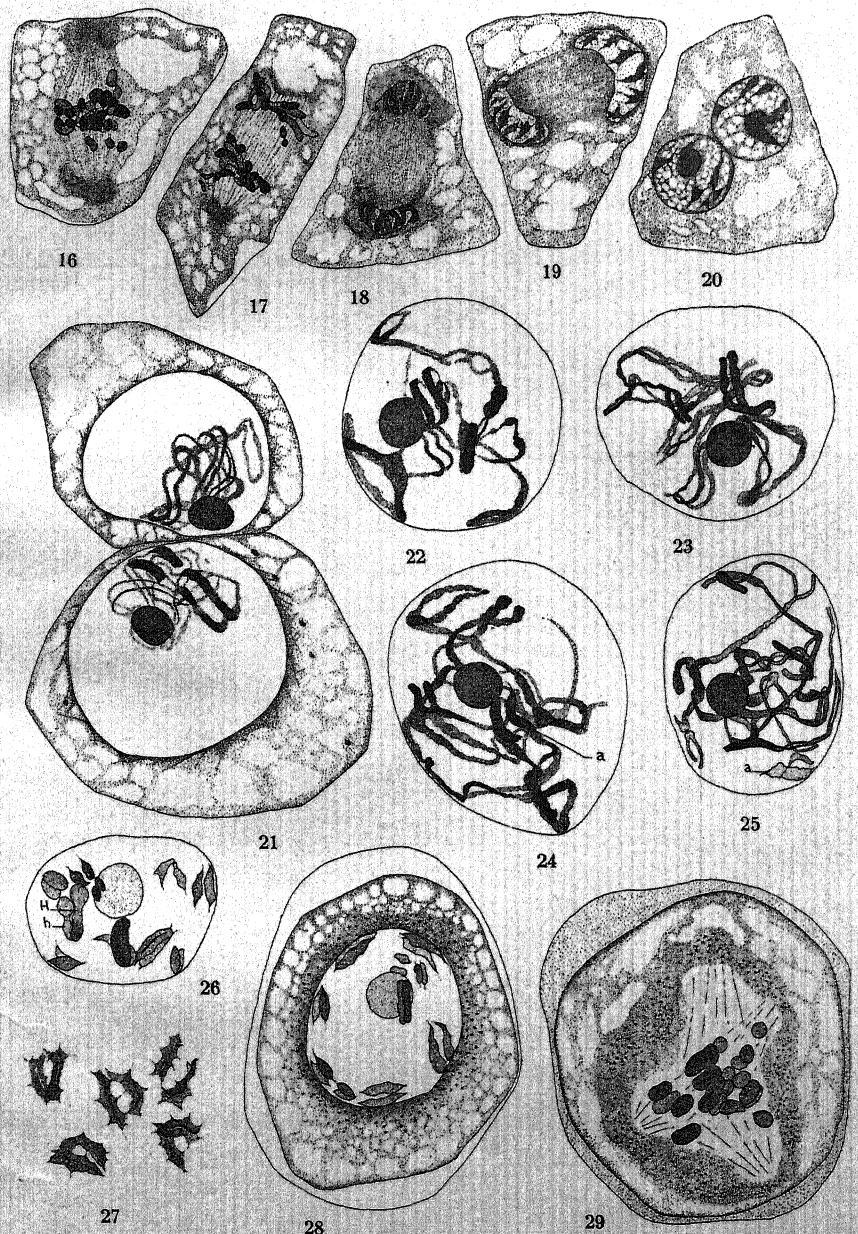
FIG. 71. Macrogametophyte showing seven nuclei. × 1560.

FIG. 72. An ovule in the early stages of disintegration. The cells of the funiculus and integuments have completely collapsed. × 240.

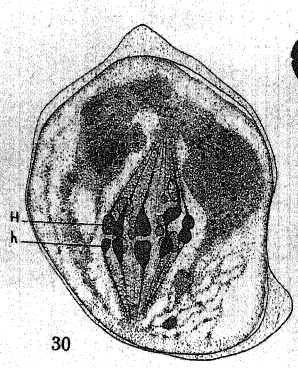


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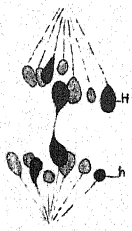
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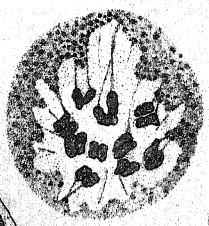
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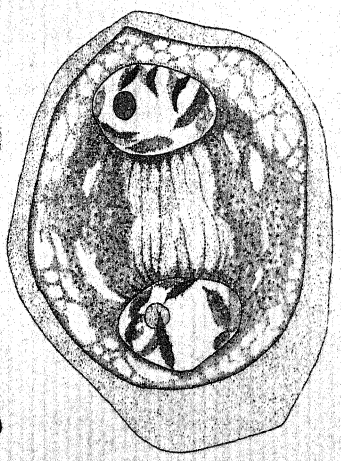
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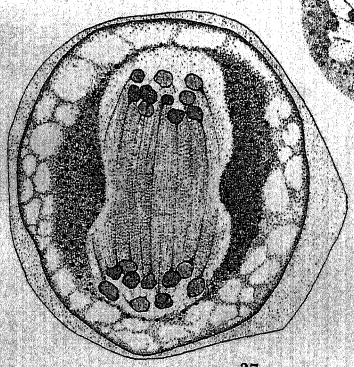
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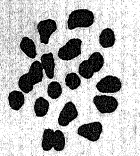
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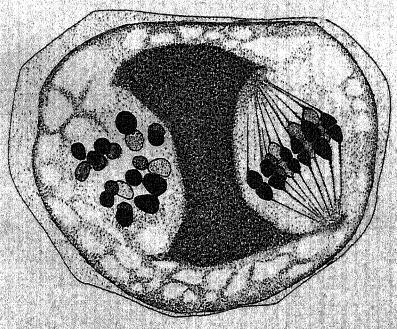
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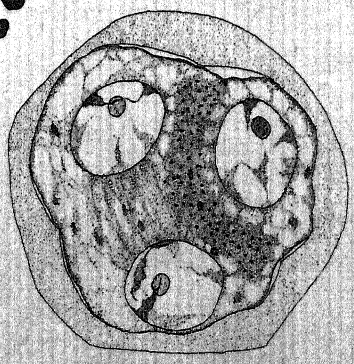
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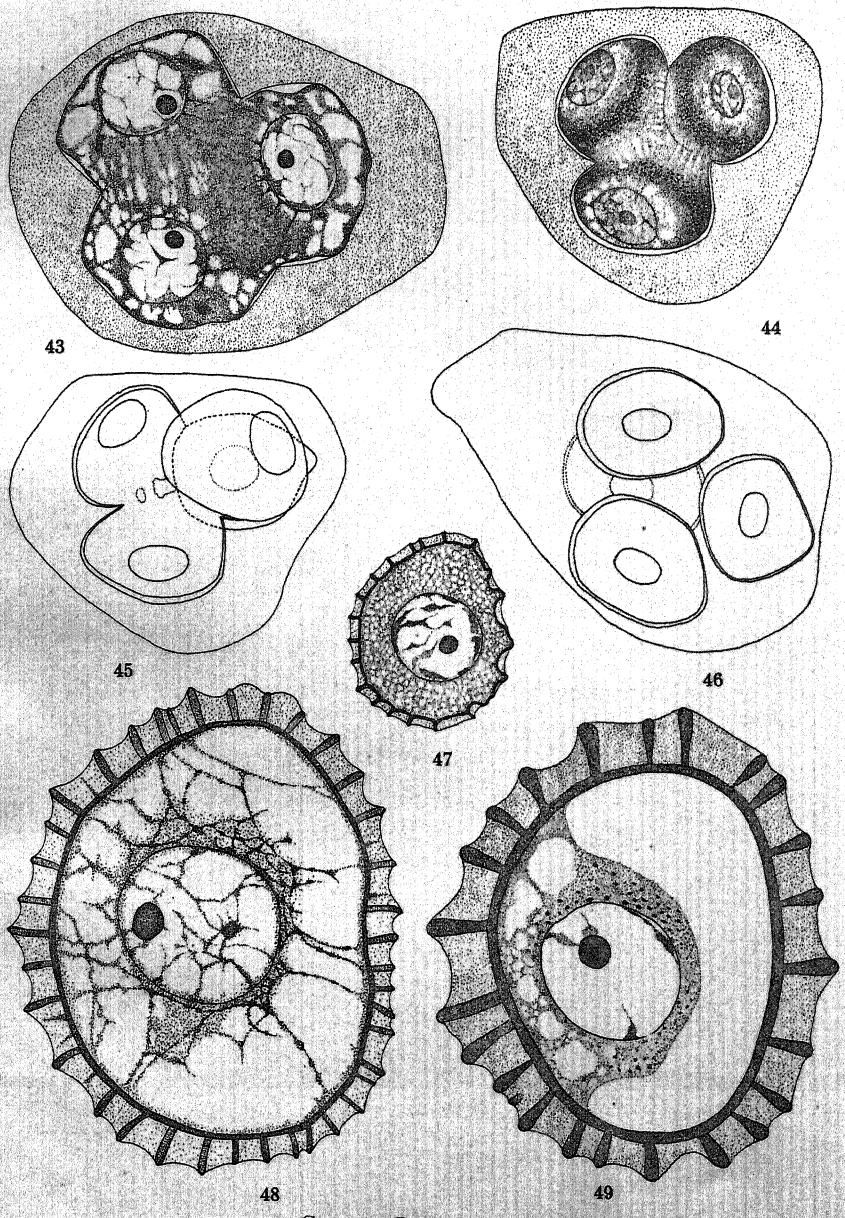


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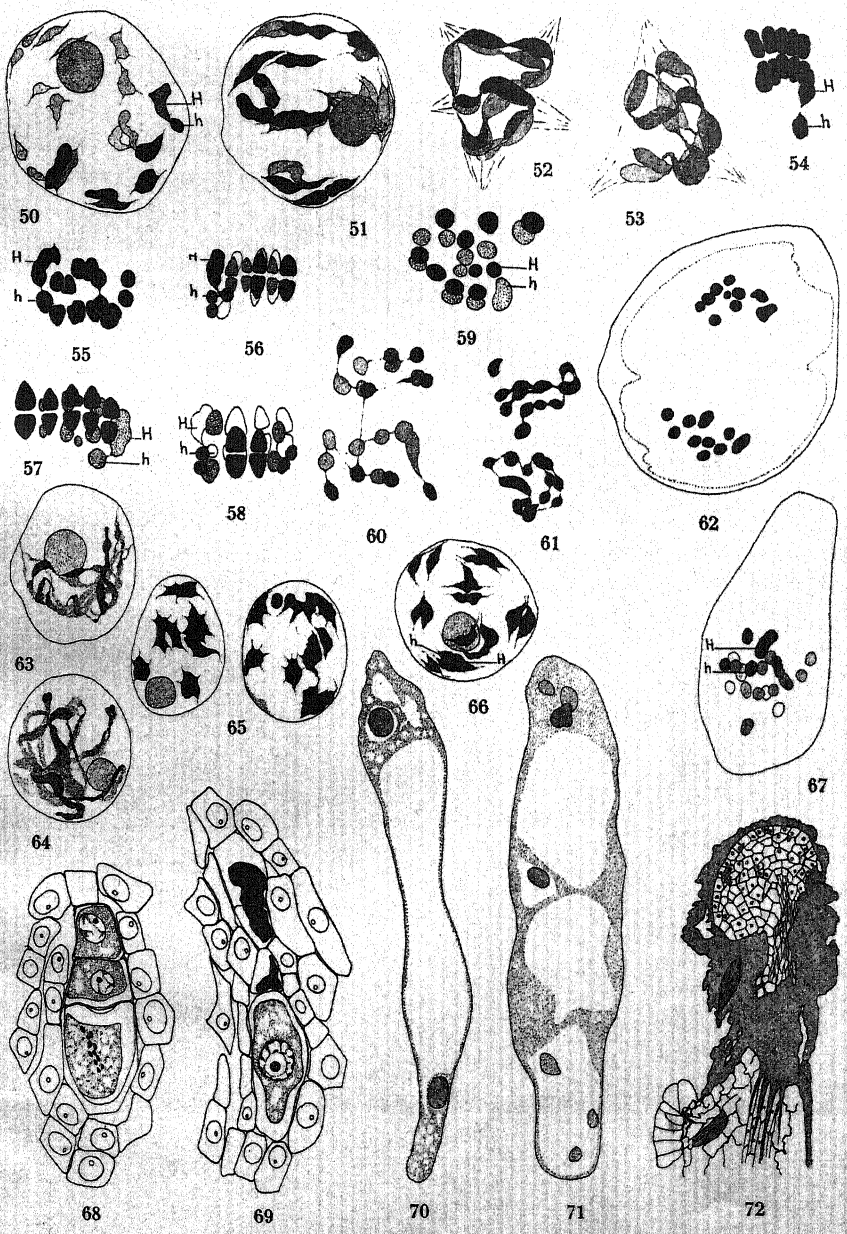


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SEX RATIO AND SEX EXPRESSION IN THE CULTIVATED CUCURBITS

THOMAS W. WHITAKER

(Received for publication December 1, 1930)

Sex expression in the Cucurbitaceae offers many interesting possibilities to the geneticist. In this comparatively large family practically every type of sex expression is represented from the dioecious species such as *Bryonia dioica* to those with hermaphroditic flowers represented by one species of *Melotheria*. According to Yampolsky (1922) the monoecious expression is the most common, the dioecious next, with hermaphroditism very rare.

During the course of a genetic investigation with the various species comprising the cultivated Cucurbitaceae, the writer has had under observation numerous commercial varieties. In connection with the other aspects of the investigation records were kept upon the sex expression of the different varieties. It was also thought desirable to learn something of the sex ratio, or the ratio of the staminate to pistillate flowers, and to obtain a record of the seasonal distribution of these flowers.

This type of work is of particular interest at present in view of the recent theory of Correns (1928) as to sex determination in plants. Data upon the sex ratio and sex expression of some of the different groups of plants which have not been thoroughly investigated from their sex standpoint will tend to bring out more clearly the merits or demerits of this theory.

As this work is primarily concerned with sex expression it may be well to define at this time exactly what is meant by the terminology employed in designating a particular type of sex expression:

1. *Hermaphroditic:* All the flowers have both staminate and pistillate parts functional.
2. *Monoecious:* Part of the flowers are staminate and part are pistillate.
3. *Gynomonoecious:* Part of the flowers are hermaphroditic and part are pistillate.
4. *Andromonoecious:* Part of the flowers are hermaphroditic and part are staminate.
5. *Trimonoecious:* There are three kinds of flowers: staminate, hermaphroditic, and pistillate.
6. *Gynoecious:* All flowers are pistillate.
7. *Androecious:* All flowers are staminate.

This is the terminology used by most authors and can be regarded as standard for the different expressions.

Historical

Heyer (from Correns, 1928) as early as 1884, working with the sex ratio in cucumbers and pumpkins (monoecious), found that the proportion of staminate to pistillate flowers could be materially changed by environmental conditions. By growing cucumbers in garden soil, he was able to increase the percentage of staminate to pistillate flowers 15 percent as compared to plants grown in the greenhouse. When growing the plants in sand he found the percentage of staminate flowers increased 20 percent as compared with those grown in the greenhouse. In pumpkins by the same procedure he was able to increase the percentage of staminate to pistillate flowers by approximately 7 percent in each case.

Rosa (1928) as a result of crossing monoecious with andromonoecious varieties of *Cucumis sativus*, *Cucumis Melo*, and *Citrullus vulgaris* concluded that the monoecious expression is dependent upon a single dominant factor in all three species. In conjunction with this work he presents data to show that the sex expression in these three species is remarkably constant. He suggests the possibility of evolution by dominant mutations as he considers the monoecious expression to have arisen directly from the andromonoecious. This suggestion is open to question, however. Correns (1928) who has reviewed the subject with considerable thoroughness represents the evolution of sex expression in the following manner:

Original form	Hermaphroditic		
Intermediate form	Andromonoecious	Trimonoecious	Gynomonoecious
End form	Androecious	Monoecious	Gynoecious

This scheme of the evolution of sex expression, indicating that andromonoecious and monoecious expressions arose from a common stem, seems to be more logical than the suggestion that the monoecious expression arose directly from the andromonoecious by a dominant mutation. The fact that instances of dominant mutations are rare in both plant and animal kingdoms casts further doubt upon the suggestion of dominant mutations.

Tiedjens (1928) succeeded in materially changing the ratio of staminate to pistillate flowers in the cucumber by increasing and reducing the amount of light to which the plants were exposed. In an abundance of light the number of staminate flowers was increased. Under reduced light conditions the number of pistillate flowers tended to increase and the number of staminate flowers to decrease. By varying the nitrogen content of the soil and the amount of light, he increased the number of both staminate and pistillate flowers, the amount of increase depending directly upon the amount of light to which the plants were exposed. The degree to which

the sex ratio could be shifted was also dependent upon the genetic potentialities of the variety. There are well marked differences between individual varieties in their ability to produce staminate and pistillate flowers under ordinary conditions.

By severing the pistillate flowers from the plant at the time of anthesis, Tiedjens succeeded in producing a 100 percent pistillate plant toward the end of the experiment.

Erwin and Haber (1929) determined the sex ratio for one variety of *Cucurbita Pepo* var. Acorn. They found that one pistillate flower was produced to fourteen staminate flowers.

Observations

The data on the ratio of staminate to pistillate flowers were taken so that they covered as nearly as possible the height of the flowering season. In counting the flowers an attempt was made not to interfere any more than was necessary with the normal condition of the plant. In order to accomplish this end with the least difficulty, the staminate flowers were entirely severed from the plant at the time of anthesis, whereas the pistillate flowers were tagged and allowed to remain on the vine.

To secure as nearly as possible uniform environmental conditions, the records were kept upon four or five plants from one hill during the entire season. In the main the plants of one variety do not vary noticeably in the ratio of flowers produced, so it was thought unnecessary to keep individual plant records.

Table 1 gives the results on sex expression.

Table 1. *Sex Expression in Eight Species of Cucurbits*

Species	Typical Sex Expression	Varietal Exceptions	Sex Expression of Varietal Exceptions
Cucumis sativus	monoecious	Lemon Cucumber	andromonoecious
Cucumis Melo	andromonoecious	Mexican Banana	monoecious
Cucumis Anguria	monoecious	—	—
Citrullus vulgaris	monoecious	Siberian Sweet New Winter	andromonoecious
Lagenaria vulgaris	monoecious	—	—
Cucurbita moschata	monoecious	Japanese Chirimen	occasionally has hermaphroditic flowers
Cucurbita Pepo	monoecious	Long Island Bush	observed 1 hermaphroditic flower on inbred strain
Cucurbita maxima	monoecious	—	—

The following list records the varieties of each species which exhibit the typical sex expression.

Cucumis sativus. Short Green Gherkin, Japanese Climbing, Everbearing, Henderson's, Long Green.

Cucumis Melo. Salzer's Earliest Ripe, Rocky Ford, Kin Makua, Heart of Gold, Lake Champlain, Henderson Bush, Montreal Market, Vine Peach.

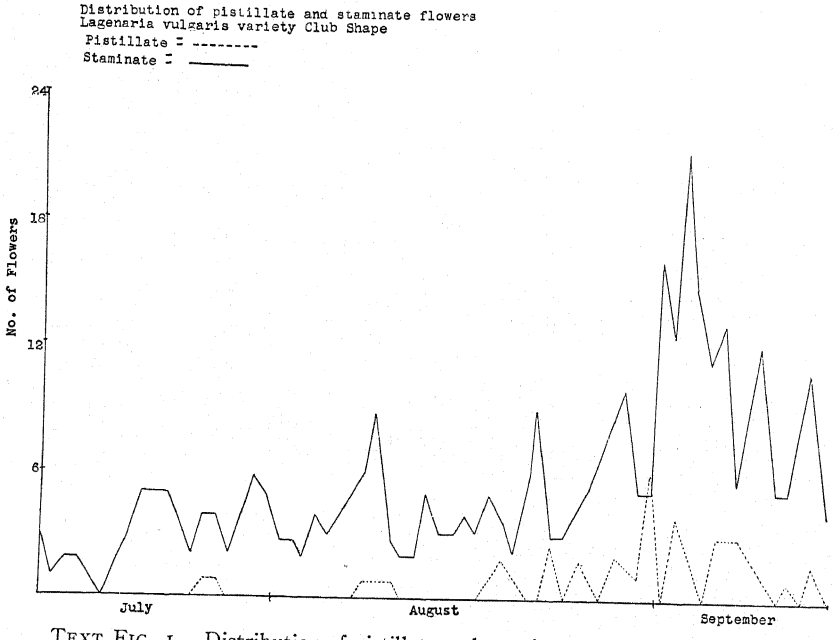
Citrullus vulgaris. Citron, Sun Moon and Stars, Tom Watson, Ice Cream, Golden Heart, Radio, Mountain Sweet, Florida Favorite, Coles Early.
Lagenaria vulgaris. Hercules Pepo, Giant Bottle, Club Shape, Zucca, African Pipe.
Cucurbita Pepo. Acorn, Delicata, Winter Luxury, Sweet Potato, Vegetable Marrow, Yellow Crookneck, Golden Custard, Fordhook, Mammoth Tours.
Cucurbita moschata. Japanese Pie, Calhoun, Large Cheese.
Cucurbita maxima. Faxon's Brazilian, Delicious, Hubbard, Golden Hubbard.

Text figures 1–3 show seasonal distribution in a variety of each of three different species.

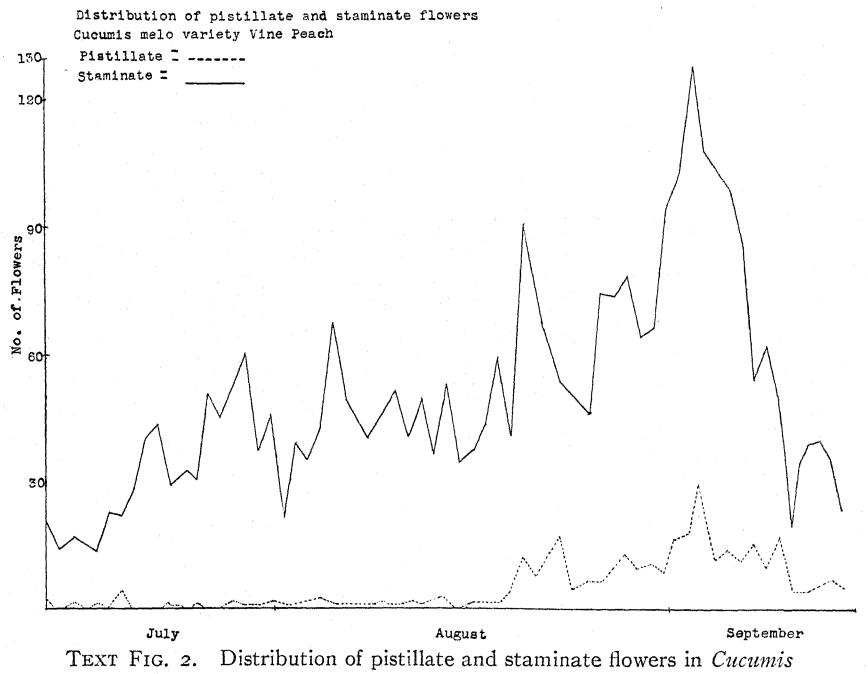
Table 2 presents the results of the flower counts in several varieties.

TABLE 2. *Flower Counts in Cucurbits*

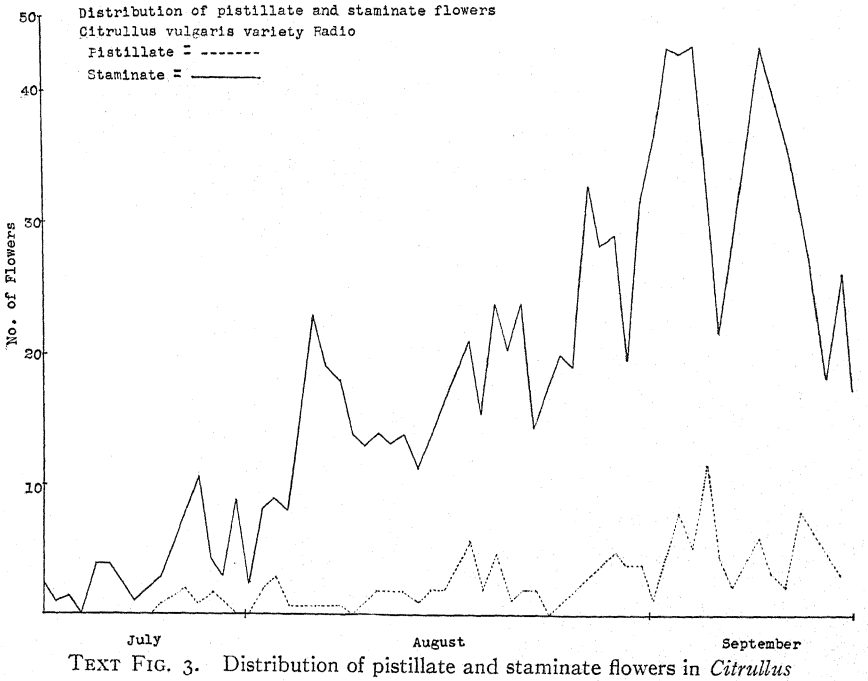
Species	Variety	Number of ♂ Flowers	Number of ♀ Flowers	Total No. of Flowers	P.E. of Total Observation	Sex Ratio
Cucumis Melo	Vine Peach	3162	372	3534	±.002	8.5 : 1
" "	Lake Champlain	1386	143	1529	±.005	9.6 : 1
" "	Henderson Bush	1221	53	1274	±.001	22.4 : 1
Citrullus vulgaris	Radio	1105	276	1381	±.003	13.8 : 1
" "	Sun, Moon and Stars	262	51	313	±.015	13.8 : 1
" "	New Winter	798	75	873	±.006	10.6 : 1
" "	Citron	239	21	260	±.011	11.1 : 1
Lagenaria vulgaris	Club Shape	349	48	397	±.011	7.5 : 1
Cucurbita moschata	Calhoun	189	33	222	±.016	5.8 : 1
" "	Large Cheese	159	15	174	±.014	10.9 : 1
Cucurbita Pepo	Long Island Bush	388	68	456	±.011	5.8 : 1



TEXT FIG. 1. Distribution of pistillate and staminate flowers in *Lagenaria vulgaris* var. Club Shape.



TEXT FIG. 2. Distribution of pistillate and staminate flowers in *Cucumis Melo* var. Vine Peach.



TEXT FIG. 3. Distribution of pistillate and staminate flowers in *Citrullus vulgaris* var. Radio.

Discussion

Correns's theory that all plants fall into four classes as far as their sex expression is concerned has given additional impetus to the study of sex expression in the various plant families. Using his classification the cultivated cucurbits obviously fall into Type III. This type includes the heterosporic ferns and the monoecious flowering plants. In this group sex determination does not take place during the reduction division but earlier, during the diploid stage.

If Correns's plan of sex determination be represented by a formula in which *A* is the gene complex of the androecium, *B* the gene complex of the gynoecium, and *Z* the gene or set of genes which determine the sequence in which *A* and *B* become activated, then the diploid formula of the cultivated cucurbits would be *AABBZZ*. The haploid would be *ABZ* for both the pollen grain and the embryo sac because this is a monoecious group.

It is recognized that *A* and *B*, the set of genes which represent the staminate and pistillate structures, are relatively stable, while *Z*, which represents the set of genes which may be affected by the environmental factors such as heat, moisture, light, soil condition, etc. may vary widely and in turn will affect the place and time development of the other two groups (*A* and *B*).

It would appear then according to this theory and in an essentially monoecious group, as the cultivated cucurbits undoubtedly are, that the only theoretical chance of altering the sex ratio is by affecting the set of genes represented by *Z*. This aspect of the question has been verified by Tiedjens (1928), as mentioned previously. The fact that he was able to shift the ratio to some extent by increasing and decreasing the amount of light and by the application of nitrates to the soil is quite in line with Correns's theory. In severing the pistillate flowers at the time of anthesis, Tiedjens was probably dealing with a phenomenon somewhat comparable to that observed by Riddle (1916) in animals. Riddle removed the eggs under the female pigeon immediately after they were laid, and thus the female was induced to continue laying. At the beginning of the experiment the eggs were half male-producing and half female-producing as the female pigeon is heterozygous for sex; but toward the end of the experiment there occurred a gradual change until all the eggs were female-producing. Returning to cucumbers, we here have a case where the gene complex represented by *A* (the androecium) is completely suppressed by *Z* and its potentialities remain unexpressed.

At present all the available evidence seems to justify the conclusion that Correns's theory of sex determination is well supported in the cultivated Cucurbitaceae.

The occurrence of marked genetic differences in the ability of different varieties within a species to produce staminate or pistillate flowers

is shown by the observations upon varieties of *C. Melo*. In this species the variety Henderson Bush has a relatively high proportion of staminate to pistillate flowers (22.4 : 1), while the varieties Vine Peach and Lake Champlain have a low proportion, 8.5 : 1 and 9.6 : 1, respectively. From these data it seems that there are well defined differences between the sex ratios in the different varieties of *C. Melo*. Such differences would indicate a diversity in the *Z* complex in the different varieties. From similar results and by hybridizing experiments it should be possible to place the inheritance of sex ratio upon a factorial basis.

The graphs of seasonal distribution show that the staminate flowers are greatly in the majority at all times of the flowering season. In most cases the staminate flowers appear a week or so before the first pistillate flowers. These two facts probably account in part at least for the extremely heterozygous condition of the Cucurbitaceae.

As one would expect, the graphs indicate that the number of flowers rises to several peaks during the season, with a gradual diminution in the numbers after the maximum has been reached. The peaks of both staminate and pistillate flowers show some degree of correlation as a casual inspection of the graphs will indicate. The sharp declines during the season are believed to be caused by environmental factors, chiefly temperature, although there are no statistics covering this point.

From the practical viewpoint the possibility that the sex ratio can be altered considerably by certain environmental conditions exists. To alter the ratio enough so as to make it of commercial value by the application of fertilizers may seem somewhat improbable, but the possibility that some form of environmental control of sex expression in this group might be of practical value is by no means beyond the bounds of probability.

Summary

1. Studies of sex expression in 49 varieties of cultivated Cucurbitaceae are considered in this paper. These varieties are distributed among 8 species and 4 genera.

2. Each species is characterized by a specific qualitative type of sex expression. In some species, in addition to the characteristic type, there are varieties qualitatively different from this type. *Cucumis Melo*, in most of its varieties, is typically andromonoecious, but the variety Mexican Banana is monoecious.

3. Within a given species quantitative differences in sex expression may exist between its varieties, this being expressed in the ratio of staminate to pistillate flowers produced over a given period.

4. Staminate flowers are greatly in the majority at all times in all thə forms studied.

5. There exists some evidence that a form of environmental control of sex expression in these forms may be possible and practicable in the future.

6. Sex determination in the forms studied appears to follow Correns's theory as applied to the monoecious flowering plants.

The writer wishes to express his thanks to Dr. O. E. White for advice and criticism.

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CHROMOSOME STUDIES ON *GASTERIA* III. CHROMOSOME STRUCTURE DURING MICROSPOROGENESIS AND THE POSTMEIOTIC MITOSIS [1]

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INTRODUCTION

The writer began regular collections for a study of chromosome form in *Gasteria* in 1922 at the University of Pennsylvania, following casual earlier observations indicating that this genus was suitable for careful cytological work. The study of chromosome form through the various developmental stages came to tolerably satisfactory completion and was published (1924, 1925, 1929). As it progressed, evidences of internal structural differentiation became apparent, and a preliminary note was published calling attention to one aspect of this (1922). Accumulating material of *Gasteria* and of other plants studied in the writer's laboratory soon showed the inadequacy of this preliminary interpretation, so that a redescription of the conditions was undertaken. The structure of the chromosomes being inseparable from the changes occurring during the meiotic phases, it was necessary to secure material for a description of meiosis, which task has, from interference by other work, been but slowly accomplished. Only the comparative adequacy of the available material in character and quantity, and its preparation by distinctly improved methods, justify the publication of another description of meiosis.

It is not worth while to analyze here the various views propounded regarding chromosome structure. The literature has been growing rapidly, and has been reviewed several times. It is impracticable to separate the literature upon chromomeres and chromonemata from that dealing with synapsis and the chromosome tetrad, with both of which this present paper is also concerned. The publications of Kaufmann (1926*a*, *b*) on *Tradescantia* and *Podophyllum* report the first studies in which the writer had a direct interest, and fairly review the earlier literature. A double spiral structure is described in somatic chromosomes, and a history contrasting with that offered by Martens (1922 *et seq.*). A spiral chromonema was also found in meiotic chromosomes. Spiral structures are next reported by Kuwada (1927) also in *Tradescantia*, who finds mechanical difficulties in separating

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the double spiral present. As will be seen by the account to follow, no such difficulty arises in *Gasteria*. Kuwada also discounts Kaufmann's description of double anaphase spirals. Disregarding theoretical interpretations offered, the writer can confirm Kaufmann's published observations from inspection of his beautifully prepared material, and is confident that optical illusions have not significantly contributed to the image recorded. Sakamura (1927) experimentally confirms, principally upon *Tradescantia*, the normal nature of this spiral structure in chromosomes, and shows under what conditions it may be demonstrated. Maeda (1928, 1930) in *Lathyrus* and *Vicia* also finds spiral structures. It appears that in *Lathyrus* the spiral form is clearly retained through interkinesis. Newton (1927) working with *Tulipa* gives a most acceptable account of meiotic prophases from the standpoint of synapsis and the development of the chromosome tetrad, but he recognizes the spiral elements only in the second prophases, at which time, though clear, they are comparatively uninteresting. A posthumous paper with Darlington (1929) also concerns itself with synapsis and the formation of tetrads or their equivalents. Babcock and Clausen (1929) report spiral chromonemata in *Crepis* and relate these to the tetrad. They record a contraction of the prophase threads to form the spiral chromonemata before diakinesis, an observation of interest since this period is obscure in *Gasteria*. Shinke (1930) surveys the list of plants showing spiral structure, making several additions, generalizes somewhat respecting the behavior of the threads through the meiotic prophases, and offers good evidence for the double nature of the thread in *Tradescantia*. He finds spiral structures in the chromosomes of the first pollen grain divisions of three genera. In vegetative cells a similar spiral structure has been reported by Kaufmann (1926*a*) and more recently by Sharp (1929), who discusses the literature and gives a very complete account of conditions in *Allium*, *Trillium*, *Vicia*, and *Podophyllum*. Similar structures and development occur in *Gasteria*, but the account of the writer's observations on that score are not ready for presentation.

Contrasted with the spiral chromonematal accounts of mitosis and meiosis are those based on chromomeres as exemplified by Belling (1928) and that involving the longitudinal cleaving of relatively condensed chromosomes with the consequent cutting of any transverse or spiral structures as represented by Martens (1922, 1925, 1929). Many of the structures which they found appear in the preparations of the writer, and the differences in interpretation will be sufficiently evident from a comparison of the reports.

First Maturation Division

I. Prophases

In *Gasteria* the resting period between the last archesporial division and the first meiotic prophase is a long one, during which there is considerable increase by growth in the bulk of the cells. Consequently there is little or

probably no chance of confusing telophasic stages of the last archesporial division with the prophases more properly under observation. In cells which have reached full growth the nuclei are about 12.2 μ [2] in diameter, nearly round, generally with one nucleolus, although up to a third of them have one or two additional nucleoli. The nucleus shows a uniform structure of dense, smoothly coagulated karyolymph, in which are embedded very many small granules of the order of 0.2–0.3 μ diameter, evenly distributed throughout the mass. These granules, while darker than the supporting matrix, do not take on a sharp chromatin stain. No definite connecting system is recognizable between them.

With advancing orientation of the nuclear contents for division the granularity becomes progressively emphasized, because of the appearance of elements which are able to hold the chromatin stains very strongly. The smallest of them are no larger than the granules seen during rest, but gradations to larger elements 0.5–1.0 μ in diameter are common. There is here also, even in the cells which have received most directly the action of the osmic acid, very little evidence of orderly interconnection of the granules. Occasional more oblong dark-staining elements appear. However, where the karyolymph is less solidly coagulated there is a strong suggestion of filaments connecting the granules with each other. This is to be interpreted as indicating that even back during the archesporial period of rest an actual though obscure system does exist anticipating the definite leptonema, not susceptible of direct demonstration in undisturbed form by the present technique, but betrayed by distorted strands imperfectly preserved when there is partial fixation of the karyolymph. There is little direct evidence that these granules are closely associated in simple strands, although it is clear that they may be connected in some fashion. The nucleus has meanwhile increased slightly, to about 14 μ in diameter.

The succeeding changes are concerned with the increasing definiteness of the spireme. Unfortunately the cells have now reached the point, for *Gasteria*, where synizetic contraction may occur, and the misleading effects of this phenomenon must be carefully discounted until, after synapsis, the cells cease to be subject to this disturbance. The general staining power of the granules decreases, so that some stand out sharply from the rest. Short bits of strand appear (Pl. XXVII, fig. 1). These become more marked and shortly in exceptionally favorable instances it is evident that the early leptotene structures which hold the stain are spiral (Pl. XXVII, fig. 2). However, they are so closely placed and so subject to lateral fusions and distortions with inaccurate fixation as to be very hard to detect, and the false impression received is that of an involved tangled thread

[2] Measurements were averaged from a number (at least 10) of characteristic cells in sections, usually of extruded microsporocytes, and usually from more than one fixation batch. They are given only as approximations to show relative sizes under the conditions of this study.

system. No chromosome vesicle or membrane was recognized as surrounding each spiral. The next stage involves a very rapid shortening of the spireme, the opening out of the spirals, and the transition to an interwoven thread system, which, as later leptonema, persists until toward the climax of synizetic sensitiveness. At mid-synizesis the tapetum cells are usually binucleate, and there is little or no degeneration evident in their cytoplasm. Occasional tapetal mitotic figures are evident. At the time the nucleus becomes subject to synizesis it is about 14.9 μ in diameter, and from this it grows rapidly to 17.7 μ or more before synapsis becomes well advanced.

This very important feature is hard to observe in *Gasteria*, but nevertheless the writer feels confident that it occurs by the passing of the leptotene strands into parallel relation in pairs. It is not difficult to find examples of short parallelisms in leptonema, but these alone are unconvincing. Favorable cases of amphinema, while uncommon, show a synapsis partially completed, the paired strands being evident on one side of the nucleus, leading to an unsynapsed part of the spireme on the other side, where the elements in the double strand diverge to become lost among the tangled simple threads (Pl. XXVII, fig. 3). Obviously synapsis is by lateral approximation of threads. It occurs while the cells are still strongly subject to synizetic contraction, and while the spireme elements are shortening very rapidly. As synapsis approaches completion, the cells lose their sensitiveness, and synizesis becomes progressively less of a disturbing factor. The synapsed spireme strands traverse the nucleus freely, and the nucleolus is still well preserved.

Up to this time there has been little evidence of definite organization within the spireme. The thread, though granular in appearance and often minutely nodulose in contour, has not shown clear-cut chromomeres. With the organization of the double spireme these elements become much more recognizable (Pl. XXVII, fig. 4). The associated threads generally show a tolerable correspondence in position, number, and size of the chromomeres over the comparatively short pieces which are in suitable position for simultaneous observation. Where the pachytene spireme strands are intimately conjoined the chromomeres may be exceedingly close together, and hard to resolve optically (Pl. XXVII, fig. 5). Free ends are definitely recognizable at this stage, though still somewhat difficult to locate because of the complexity of the system, which in its much greater involvement during earlier stages effectively prevented the forward extension of this observation (Pl. XXVII, fig. 4). There is now begun a continued rapid shortening of the spireme elements, with a tendency for long strands to lie against the nuclear wall, where they assume roughly parallel courses and seldom cross each other in close contact. Where exigencies of space might force too close a convergence one of the strands generally turns down to traverse the karyolymph. The spireme is definitely not limited to a peripheral position at this time, though much of it is peripheral, and this

actually corresponds to what has been described as the "hollow-spireme" stage. The nuclei of fixed extruded cells are about 19.6 μ in diameter. As the stage is very abundant it probably is a prolonged one.

There follows a short and rather confusing period during which the synapsed threads separate somewhat widely. This diplonema is often accompanied by an apparent tangling about the nucleolus, and frequently paired loops appear to radiate from the tangled mass (Pl. XXVII, figs. 7, 9). Sometimes the process is not simultaneous throughout a cell, and the transition is seen (fig. 7). Chromomeres, at first distinct, become less numerous (fig. 8) and ultimately less distinct. The parallelisms of the peripheral pachynema were transitory and nothing comparable is now to

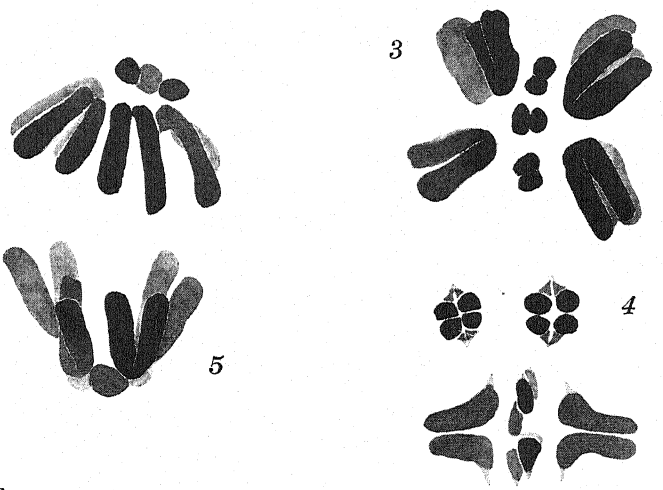


TEXT FIGS. 1, 2. FIG. 1, Mid-pachynema, threads not well enough preserved to show chromomeres and partially fused, but showing doubleness by the paired free ends. FIG. 2, Tetrads from one cell, representing three of the large chromosomes. × 2060.

be seen. As the shortening proceeds there becomes evident a considerable thickening of the threads, which was not marked during the earlier contraction.

The passage into strepsinema is rapid, but marked by no peculiar features (Pl. XXVII, figs. 9, 10, 11). Soon it appears possible to count the number of sets of elements present, though actually this can rarely be done with accuracy, since there is much overlapping (text fig. 1). In the best-placed bits of strand it is also possible to recognize a lateral segregation of their substance, with in effect the establishment of chromosome tetrads (Pl. XXVII, figs. 10, 11). At first this is evidenced by occasional short splits and seeming openings in the threads. Probably the continuity of the chromosome membrane is not altered by these, if indeed the tetrad has yet been established as a definite structure (Pl. XXVII, fig. 10). Later, as the strepsitene elements become shortened, definite separation shows two longitudinal chromonemata (Pl. XXVII, fig. 11). There is usually some slight spiral twisting, and very generally vacuolization of the matrix. Physical isolation of the chromonemata into separate chromatids is not immediately effected, but a well-marked tetrad is, though briefly, present (Pl. XXVIII, figs. 1, 2, 3; later, text fig. 2). The extruded cells at this stage have nuclei of about 17.1 μ diameter. The shortening does not

proceed to the extent of the formation of peripherally placed chromosome pairs in typical diakinesis before the breakdown of the nuclear membrane. Rather, the chromosome elements lie in the cavity, often with some tendency to clump in a vague contraction until the quick drawing in and dissolution of the nuclear membrane frees the chromosomes. During the period of crowding contraction of the tetrads is completed, the chromonemata each being apparently thrown into a spiral in its contracting chromatid. They are thus isolated in separate bodies which are at first closely placed side by side, but which separate as metaphase is established. Obviously, even if



TEXT FIGS. 3–5. FIG. 3, Polar view of metaphase plate to show the separation of the sister chromatids. FIG. 4, Lateral view of metaphase plate, two homologs, displaced for clearness sake, showing the tetrad structure of the homologous pairs. FIG. 5, Side view of anaphase figure. × 1470.

the process is a coiling one, each thread coils freely and separate from its neighbors, so that no difficulty due to interlocked parallel spirals exists here, in contrast to several other accounts, where it seems necessary to postulate some method of disentangling the involved coils. At first crowded together, with the dissolution of the nuclear membrane the chromosomes later spread out in the plate position, three small pairs near the center and four large pairs radiating therefrom (text fig. 3).

II. Metaphase

The lesser metaphase chromosomes are small rounded oval structures, somewhat elongated in the spindle axis, and slightly compressed parallel to their axes (text figs. 3, 4). Often it is difficult to distinguish more of their true constitution at metaphase, but from polar view it is generally clear that the compression is more fundamental than appears from the side, and one sees a fairly marked cleft on each face (text fig. 3). The larger

metaphase chromosomes are elongate, generally about 4 diameters long. One is from the first inclined to be bowed from the plane of the plate, the rest lying more closely side by side (Pl. XXVIII, fig. 4). All when viewed from the pole are definitely divided into closely placed chromatids, so that when viewed from the end each pair of homologs is unequivocally tetrad in its appearance as well as its constitution (text fig. 4).

The inner or spindle ends retain closer association than the outer ends, but are essentially separate structurally, however closely placed they may appear. When spindle fibers become recognizable it is found that one thickened 'tractor fiber' serves for each 'diad' pair of chromatids, large or small (Pl. XXVIII, fig. 4). On all but the one bowed pair of large elements the attachment is closely terminal, but on that one it is atelomitic, behind the tip, and related to the bowing of the element. As is indicated by their contour, and in the light of what has become known of their internal structure, the smaller chromosomes may be confidently assumed to be structurally divided at this time like the larger ones.

During the extreme concentration of the chromosomes when first freed from the nuclear membrane and because of the density of their interior, nothing has been clearly determined as to their structure by direct observation. When the plate has been organized for separation of the homologs conditions are very much changed. While the chromosomes are still extremely dense, it is easily established by critical differentiation in destaining that a distinction exists between the substances of which they are composed. At first only betrayed by the occasional crenellate appearance of the apparent margin, each is differentiated with further destaining into a dense core or matrix and a peripheral system resembling transverse denser rods (Pl. XXVIII, fig. 4). An accurate interpretation of most of the examples would be difficult were it not possible to work back from subsequent stages, and also were it not for an occasional more intelligible instance. In these favorable cells it is seen, and may be confirmed, that what at first look like transverse bars about the core, or like larger or denser disks alternating with smaller or less dense ones, really are turns of a spiral rod which winds about the chromatid or univalent metaphase chromosome at its periphery (Pl. XXVIII, fig. 4 p.p.).

Meanwhile, the spindle has been organized. Because of the character of fixation employed, which coagulated even the dilute karyolymph, the cytoplasm is so densely and evenly coagulated as to show comparatively little differentiation between the peripheral and spindle areas. However, the exterior region is definitely and minutely spongy in aspect, with no directional trend to its delicate reticulations, while the central or spindle region shows a striation roughly parallel to the axis of division even though it is equally smoothly fixed with the peripheral portion. This spindle ultimately assumes a biconical form. In conjunction with the chromosomes themselves a marked differentiation does, however, occur. At the apparent

traction point a denser structure appears, usually serving to attach the two chromosome halves, sometimes furcate, but single above and gently tapering off to merge with the more delicate spindle elements. In fixations with reduced osmic-acid content these attachment bodies terminate markedly heavy traction fibers, notably thicker than those of the bulk of the spindle, but where fixation is more complete and uniform and the spindle does not stain differentially with respect to its constituent parts, the distinction is harder to establish.

III. Anaphases

The first sign of activity on the plate may be marked by the spreading apart of the outer ends of one of the tetrad chromosomes (Taylor, 1929), with separation along the secondary split, not along the synaptic plane. Previously the halves of the tetrad have lain side by side, or bowed apart like stomatal guard cells, with the separation along the synaptic plane (Pl. XXVIII, fig. 4). The inner or spindle ends then move away from the plate position, retaining the juxtaposition of the diad chromosome tips while the homologs separate. The separation of the tips seems to pull the outer ends somewhat together before they finally separate, and the diads pass up toward the poles fairly parallel again. Usually one chromosome pair passes thus into anaphase before the others become active, but the remainder then move up together without marked divergence of their outer ends. It could not be absolutely determined which chromosome behaved differently from the rest, but since only one of the larger chromosomes is morphologically distinct it is probable that this (in the vegetative division the satellite-bearing) chromosome is the one concerned. While there is close approximation of the spindle ends of the chromosomes it is certain that natural separation of the halves has been complete before metaphase and that apparent partial joining is illusory, or an artifact from poor fixation (text figs. 3, 5). The chromosomes group themselves at the pole with the smaller elements in the upper center and the distal ends of the larger ones subparallel to the spindle surface.

As the chromosomes pass into anaphase they lengthen somewhat, and become much more easily differentiated into their constituent matrix and chromonema (Pl. XXVIII, fig. 5). A definite morphological membrane about these two constituents is apparently not demonstrable in early anaphase in undamaged chromosomes, but becomes more probable later. The presence in each half diad chromosome of one continuous chromonematal rod is perfectly obvious, it making 5–6 turns, either with right- or left-handed twist. Occasionally (as illustrated) the twist reverses its direction in part of a chromosome (Pl. XXVIII, fig. 5 p.p.). This is in harmony with the belief that the chromonemata of metaphase and later chromosome stages are formed by the twisting of the post-strepsinema filaments when the tetrad of chromatid threads is contracted in the clumping and condensation accompanying the dissolution of the nuclear membrane.

There has been a shortening of the spiral from the 7–8 turns present at metaphase (even more in the bowed atelomitic chromosome). Suggestions of doubleness rarely appear (Pl. XXVIII, fig. 6). There are suggestive evidences that the proximal ends of the chromonemata are folded back for a short distance parallel to the main shaft, and this flexure may well occur at the future fiber-attachment-constriction point, the folded part representing the "head" of the chromosome (Pl. XXVIII, figs. 5, 6, 7). If, as seems probable, this flexure has a purely mechanical explanation it need not be expected in chromosomes with median attachment. An indefinite biconcave zone of denser cytoplasm may sometimes be seen between the telophase chromosome masses, but this rarely is very evident and very rarely indeed, though then clearly, was a specimen seen where a heavy pectic wall had been formed between the two daughter nuclei.

IV. Telophases and Interkinesis

From this point two descriptions must be given, representing either conditions leading through an extended period of rest, or by contrast through a brief and partial dissociation quickly followed by the homoeotypic mitosis. No fundamental difference separates them, and indeed intergrades occur, but the aspects of the extremes were notably dissimilar. The more deliberate process may be described first. The chromosomes at the pole draw close together, widen and shorten. Each chromosome group revolves its axis through 90° until it lies across that of the spindle. There is no radial correlation between the positions of the two sets, each taking up its position across the old spindle axis at 90° without reference to the radial position of the other. The chromonematal turns draw together, and temporarily become indistinct. Then the incipient nucleus begins to expand rapidly, with the nuclear membrane probably already present, while the chromosomes may remain laterally approximate. By reason of rapid elongation and lateral connections the chromonemata quickly lose their spirality, and assume an irregular angularity accentuated by the lessened difference in stain-holding power between the chromonemata and the interconnections. The chromosomal chromonemata seem to become the spireme portion of the nucleus directly, and the matrix portion with any included cytoplasm to enter into the karyolymph. The whole nucleus loses in stain holding power, and the chromatic continuity of the spireme becomes interrupted. Boundaries between adjacent chromosomes are visible for a long time, since the spireme from each chromosome is mostly toward the center of its area, the "chromosome tract." Retention of a distinct membrane between each chromosome vesicle could hardly be affirmed, but the evidence is clearly for the maintenance of the individuality of the spireme from each chromosome and the maintenance of the relative positions of each chromosome with respect to the others. Ultimately the nucleus may develop an aspect of coarse sponginess with chromaticity retained at a multitude of

apparent intersections of alveoles or threads. No single large nucleolus is developed, although one to several little round nucleolus-like bodies do appear, probably of essentially the same nature. More often the homoeotypic prophases begin before the extreme stage of dispersal with the loss of recognizable chromosomal tracts has occurred.

In the more accelerated procedure the chromosomes spread out rather widely upon reaching the pole, without making, or at most incompletely making, their 90° shift of position. A nuclear membrane then encloses the chromosome group and the adjacent cytoplasm, the latter immediately becoming more uniform in texture. The chromosomes enlarge considerably, the loops of the chromonemata increase in diameter, and become less strongly stainable. The definite boundary of the "chromosome vesicle" becomes vague, first at the distal end, and then throughout. The chromatin may be somewhat segregated in the chromonemata, giving a spotty appearance which, with the crossing of the chromonema, once induced the writer to report incorrectly that definite rows of chromomeres were present at this stage (Pl. XXVIII, figs. 8, 9, 10). The later telophasic stages were not available in this material, but the early homoeotypic prophases following it apparently may be normal. Instances have been seen, and will be reported upon by H.-C. Tuan, in which interkinetic dispersal and even telophases have been omitted, the cells proceeding directly into the second meiotic division from the first anaphase stages.

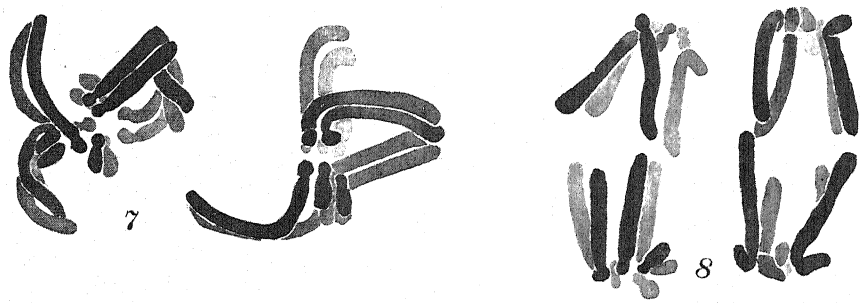
Second Maturation Division

V. Prophases

With the inception of activity the chromaticity of the nucleus again increases, and it becomes more granular in appearance under low power. The chromatin-bearing structures are mostly near the surface of the nucleus. Soon it is evident that there is a transverse arrangement of the chromatic structures, for a banding appears which, in the light of subsequent developments, may be designated as the reappearing of "chromatic tracts." A bit later very careful observation demonstrates that the stained elements tend to be longer across the axis of the tracts. Subsequently they appear to extend across them, and then it is found that they are connected together (Pl. XXIX, fig. 1). The structure thus disclosed becomes the homoeotypic chromosome by a simple condensing process. Each at first appears somewhat zig-zag, for the chromatin material is rather near the surface, but as the continuity becomes more obvious it seems to adjust itself to more regular loops (Pl. XXIX, fig. 2), and appears as a definite spiral which increases in symmetry to a marked degree (fig. 3). At first the close approximation of the several spiral elements produces an aspect of great complexity and confusion, but even then the limits of each spiral element can be traced for considerable distances (Pl. XXIX, fig. 3). As the elements shorten the

diameters of the spirals lessen somewhat and the elements become much thicker. Immediately it is possible to trace each element through its whole length one is able to recognize that the spirals always occur in pairs, closely approximated at one point (text fig. 6, Pl. XXX, figs. 1–4). The element beyond that point of juxtaposition is short or longer depending on the chromosome, for the point is readily traced through and found to develop early into a constriction which corresponds to the spindle fiber attachment constriction, and the part beyond the point is the head end of the chromosome, or the short arm according to the typical chromosome configuration





TEXT FIGS. 6–8. FIG. 6, Five diad or sister half-chromosome pairs, of successive ages, from cells in second meiotic prophases. The approximation at the point of future fiber-attachment shows, but no other structural differentiation. FIG. 7, Polar view and lateral view of two chromosome groups in second meiotic metaphase of the same microsporocyte. FIG. 8, Lateral views of two anaphase chromosome groups of the same microsporocyte. FIG. 6, × 2060; FIGS. 7, 8, × 1635.

in *Gasteria* (text fig. 6, Pl. XXX, fig. 4). The fact that the chromosomes come out of the homoeotypic prophases in the same general form as the spiral chromonemata exhibited in the heterotypic telophase, and in the same paired relation as the diads (heterotypic half chromosomes or univalent chromosomes) showed, and finally with the same polar interrelation, is one of the best possible confirmations of the continuity of the diad chromosomes in meiotic interkinesis.

To return to the early stages where continuity of the element becomes

clear: *i.e.*, as Pl. XXIX, fig. 2. It is evident that the element is of unequal thickness, and on careful examination in favorable places one notes that this is because two threads are involved, more or less parallel, and in part joined (Pl. XXIX, fig. 5*a*). In the early stages they are never regularly parallel. Later, in the continuous spiral stages the threads become too closely conjoined to permit of recognition. As the spirality is lost in late prophases the duality of each element becomes evident again in the often-compressed chromosomal structure with marginal, or at least double peripheral chromonemata (Pl. XXIX, fig. 5*b*). While the diad chromosomes remain attached at the point of future spindle attachment throughout the prophases, they diverge greatly toward the distal ends, which divergence becomes more striking as the process advances toward membrane dissolution (text fig. 6, Pl. XXX, figs. 1–4). As the prophases are completed the chromosomes are tolerably regularly distributed against the membrane, all their heads oriented toward one pole (Pl. XXX, fig. 5).

VI. Metaphase

The membrane disappears without much contraction of the mass, and the diad chromosomes draw close together into apposition, while they shorten somewhat. A general readjustment of position occurs to bring the head ends together, but now in the plate position (text fig. 7). The longer chromosomes cannot lie in the plane of the plate from limitations of space, so they curve away, some toward one pole and some toward the other, but the three short pairs lie on the plate level. Structurally the chromosomes show a condensation and increased twisting of the chromonemata, but these are by far less distinct than earlier (Pl. XXIX, fig. 5*c*).

VII. Anaphases

As separation begins the sister half chromosomes first part from each other at the constriction point, and arch apart until the head ends are separated. Then the shafts gradually separate. Ultimately the chromosomes stand straightly opposed, all the constricted ends at the poles and the heads pointing (in general) toward the axis. The length and diameter of the anaphase chromosomes varied very much indeed, being longer at first in those which are passing more slowly through meiosis. In any case the chromosomes shorten greatly as anaphase progresses. At least when they are initially attenuate they practically reach the spindle poles by the time that the distal ends have separated, so that anaphase is not so much a further passing to the pole as a contraction in length, with the head ends already in the final position (text fig. 8, Pl. XXIX, fig. 6). Structurally the chromonemata appear to straighten considerably (fig. 5*d*). This stage is harder to elucidate than the heterotypic anaphases. It approximates in structural character to the simple vegetative anaphases. Frequently the chromosomal core is much vacuolate and generally the stain is very firmly held throughout.

VIII. Telophases

When the contraction is complete the chromosomes draw somewhat close to the spindle axis and become enveloped by the new membrane (Pl. XXIX, fig. 7). The new nucleus then enlarges considerably, particularly in length. The chromosomes lengthen greatly, but do not take on a markedly spiral form, lying nearly straight against the nuclear wall with the chromonemata side by side as parallel bands (Pl. XXIX, figs. 8–10). Dispersion of the chromosomes is not rapid, but takes the course of gradual separation of the chromonemata, with a gradual loss in stain retention power of the chromatic part, so that the strands become pale and diffusely granular (Pl. XXIX, fig. 10). The chromosomal outlines become vague; the aspect of a greater density between the chromonemata disappears so that they seem to be free against the nuclear membrane unconfined by a chromosomal vesicle. By the time cytokinesis is complete it is difficult to designate the limits of the individual chromosomes in each nucleus.

IX. Cytokinesis

After the completion of the nuclear membranes there is established a fibrillar texture in the cytoplasm, radiating from each nucleus and connecting each with its neighbors. This shows best where the osmic acid of the fixing fluid has not done its work well. A clearing of the cytoplasm occurs midway between the opposite nuclei, the planes intersecting near the center. Centripetal cleavage occurs rapidly along these planes, followed by a slight rounding and separation of the protoplasts, especially near the center of the group. Then a membrane grows in, continuous with the gelatinous envelope about the whole quartet. On the completion of this the changes become more deliberate in the establishment of the young pollen grain.

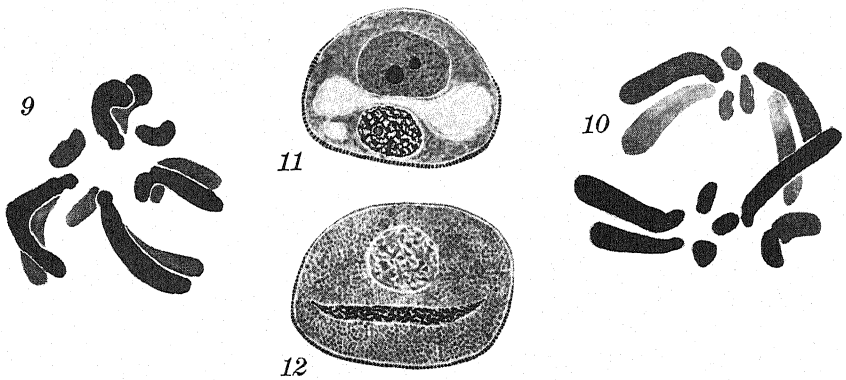
The peripheral cytoplasm of each cell becomes quite dense as a thin layer, and inside the gelatinous wall a thin, new membrane is formed. After this the gelatinous envelope progressively disappears. The chromatic elements of the nucleus complete their dispersal and the resting stage of the young one-nucleate pollen grain shows a spherical nucleus with irregularly granular contents and a large nucleolus. While some of the granules are moderately conspicuous they do not correspond to the number of chromosomes later formed.

XII. First Gametophytic Mitosis

Preservation of the contents of the *Gasteria* pollen grain by the haematoxylin smear method was very much superior to that secured by any other. Although the quality was not at all equal to that found in root tip cells, it gave a far more normal picture than usually presented by the sparse figures and descriptions of pollen grain mitoses that have been published, and so it seems worth while briefly to describe the process.

With beginning activity the diffuse granularity gives way to more coarse

granules, and these to irregular elongate strips of chromatic structure, the beginning of the spireme (Pl. XXX, fig. 6). Even with the somewhat imperfect fixation imposed by the heavy chitinous wall acting as a hindrance to penetration of the fixing fluid, the beginning spireme shows the duplex structure it inherits from the second meiotic anaphase. The continuity of the spireme strands becomes more evident and their contours more even, although they are much interconnected by anastomoses (Pl. XXX, figs. 7, 8). At first they are more or less twisted in form, or even spiral (Pl. XXX,



TEXT FIGS. 9–12. FIG. 9, Polar view of metaphase in first pollen grain mitosis. FIG. 10, Oblique lateral view of anaphase in first pollen grain mitosis. FIG. 11, Young pollen grain with beginning differentiation of nuclei. FIG. 12, Pollen grain with approximately mature nuclei.

figs. 9, 10, 11); later they become simplified to looped or bent rods, by which time the double character is obvious. The distinction between the large and small elements is finally very easy, and ultimately the nucleolus disappears (Pl. XXX, fig. 12). The small chromosomes generally lie together in the nucleus. With the breakdown of the nuclear membrane the chromosomes quickly form a very briefly maintained metaphase plate and then pass through the anaphase separation without a marked spindle (text figs. 9, 10). No internal structure was demonstrated in the chromosomes, but they showed shapes characteristic of the genus and most nearly like those of the second meiotic anaphase. However, this is, except for the absence of the distal satellite, practically the same as for the sporophytic vegetative chromosomes. There appeared nothing of note in the sparse telophasic cells seen. At first the two daughter nuclei are similar in appearance. Later they become separated by a vacuole, and the generative nucleus becomes granular, with sharp chromatic contrast, the tube nucleus more evenly and densely granular (text fig. 11). Finally, the generative nucleus elongates and the tube nucleus becomes less dense, while the cytoplasm fills up with stored food, displacing the vacuole and preparing the cell for transfer to the stigma and subsequent development there (text fig. 12).

Discussion

It is well to outline briefly the nature of the material which was used in this study, and the treatment accorded it. Greenhouse grown plants of several species of *Gasteria* were utilized, and no distinction was found in so far as this study was concerned. Where adequate the smear method developed by the writer was employed, with modifications suggested by Kaufmann (1927) and by Tuan (1930) after they became available. Because of the complexity of the spireme during mid-prophases and the coherence of the archesporium during early prophases the method was then unsuitable. Likewise at later stages, it sometimes failed when superposition of structures confused the images. The great virtue of the smear methods is that they bring the pollen mother cells into immediate contact with the killing fluid, so that if it is well chosen it does its work, unaltered by passing through varying numbers of cloaking cell layers, with precision, without causing shrinkage, and without permitting degeneration changes to occur. Where the above-mentioned reasons enforce the use of a section method, it is necessary to approximate the conditions which give the smear method its virtues. This was accomplished as far as possible for the present study by placing the androecium in a small quantity of the fixing fluid in a small wax-bottomed dish and immediately dissecting it as minutely as possible with very fine and sharp scalpels, dividing each anther into as many fragments as humanly possible within the few moments permissible. All of the fragments of a given fixation were assembled by pipette in a vial and handled by sedimentation through the succeeding stages, which in general involved close stages of alcohol concentration at short intervals, followed by alcohol-xylol intergrades, careful washing with well-dried xylol and as brief infiltration in paraffin at as low temperature as would give complete support. This mass of minute fragments yielded many small clumps of pollen mother cells extruded before they were affected by the fixing fluid, many isolated cells smeared over the anther surfaces, or anthers sliced open to yield areas of cells brought immediately into contact with the fixing fluid by the passage of the knife. Only such cells as had thus been immediately exposed to the fixing fluid were considered to afford reliable evidence for this study. Comparisons of cells in the center of an extruded mass, or in the center of an anther, with those at the surface of the mass or on the cut end of the anther abundantly confirmed the wisdom of this choice in the more perfect coagulation of the karyolymph, more precise contours of the spireme and chromosomes, their more regular and appropriate distribution in the space available to them, and their more intelligible internal structural differentiation. The fixing fluids used were various, but particularly modifications of Flemming's chrom-osmic-acetic solution with maltose and urea, which proved by far the most satisfactory. It is under just the circumstances provided here, with direct contact between cells to be studied and the fluid, that mixtures based on osmic and chromic

acids do their best work. The writer submits that no studies of meiosis with the mother cells enveloped by anther or sporangium tissues are likely to afford as truthful pictures as would have been had if the cells had been directly exposed, and doubts if the picture afforded under the more unfavorable conditions is often even approximately correct in detail.

In a *Gasteria* anther fixed intact the diameter of the nuclei was 19.4 μ at the climax of synizesis, with the synizetic mass 9.9 μ in diameter, showing a considerable increase in the volume of the nucleus since archesporial rest —from 950 μ^3 to 3823 μ^3—while the synizetic spireme and nucleolar mass occupied but 508 μ^3 of space, showing that under the unfavorable fixation conditions producing synizesis the spireme actually contracts from the archesporial space occupied, while the nucleus has enlarged. Very roughly, the nucleus has increased more than four times in volume. The changes probably involve a considerable intake of water, with a reduced concentration and reduced coagulability of the karyolymph. The spireme in organizing during this period seems to be already shortening, and this natural tendency is probably enhanced by special sensitiveness. Without the support of a simultaneously coagulating matrix at the time of killing the contractile spireme is left to condense into a tight knot, anchored at the point on the nuclear membrane where the coagulation began with the entrance of the fixing fluid, or about the nucleolus, or both. Throughout meiosis the slowly penetrating killing fluids and those acting by dehydration allow the chromatin-bearing elements to contract, and in early prophases more than later, when the karyolymph is again more easily coagulated. Increase of the proportion of osmic acid is a help in fixing these early stages, but this substance is comparatively ineffective if it has to pass through a succession of cells to reach the ones desired, and if disproportionately increased interferes with staining.

It should be emphasized that the permanent nuclear elements of the *Gasteria* cell are a number of threads which are recognizable as spireme strands or as chromonemata during cell division, but which, from limitations of technique, are not so surely recognizable during resting periods. It is not believed that these threads become discontinuous or really lose their identity or necessarily join together terminally during rest. Chromomeres may appear on these threads, and like the threads themselves may assume or lose the power of holding dyes. Irrespective of stainability, it is the chromonemata rather than the matrix or the membrane of the chromosome which are the parts definitely retained for transmission in division. Morphological differentiation of the threads is established, and chromomeres may be one feature of it, but they are so liable to artifactual irregularities that they must not be unduly stressed. It is to be noted further that the chromonemata or spireme threads are always double, or paired, structures, and that each division is prepared for before the last previous division is completed by the division of the chromosomes into two contained chromo-

nemata which will organize as parallel spireme elements for the split chromosomes of the next division. It is inferred in this account that the split which separates the elements to be isolated in the second meiotic division does not occur until late in the first meiotic prophases, but it may occur in the last archesporial anaphases and be optically unresolvable until recognized much later. The split in the chromonemata which will be isolated in the first post-meiotic division is evident in the earliest second meiotic prophases, and some evidence is offered for at least the beginning of the split in the first meiotic anaphase.

The significance of the spiral chromonemata may be involved, but one simple explanation appeals to the writer as probably adequate to explain their occurrence. Beyond certain limits longitudinal contraction of the spireme threads is somehow disadvantageous. Perhaps the actual physical seriation of its parts could not then be maintained. But it is mechanically more difficult to separate long and tangled chromosomes than short ones. So the spireme elements coil up as meiotic metaphase approaches, retaining the advantages of a long chromonema and those of a short chromosome. If the chromonema divides within the chromosome, at some stage before the split must become effective the daughter chromonemata are straightened until they are able to separate. The degree to which similar appearances may be expected in other plants may in part be predicted. Large-chromosome species will show them when the technique is adequate. Small-chromosome species do not offer as suitable material, and plants with many chromosomes do not have the same need for this mechanical adaptation. However, comparable structures and a comparable history are probably widespread in plants, though variations from this account, as well as corrections to it with improving technique, may be expected.

Summary

With inception of meiosis the initial granularity of the archesporial nucleus passes into indefinite short strands which, becoming more numerous and regular, develop as a number of spirals transversing the nucleus. The strands constituting these spirals, straightening, lose their relative positions and interweave through the nucleus. They then swing together in pairs, effecting synapsis, until the whole thread system is a double one. Chromomeres have appeared on the thread, and are generally found in similar pairs, but gradually again become indistinct. The paired threads, continuing to shorten, separate gradually from each other. It becomes recognizable that the number of pairs corresponds to the haploid chromosome number. Each element splits, forming a tetrad of four chromatids. A new shortening progresses very rapidly, and it appears that each thread is thrown into coils rather than shortened and a membrane develops about the spiral. The nuclear membrane disappears, and the metaphase plate is organized with three small homologous chromosome pairs of terminal spindle fiber

attachment, three large pairs with terminal attachment, and one large atelomitic pair. Each pair is obviously tetrad, and each chromatid contains a spiral thread or chromonema. Fourteen elements pass to each pole of the spindle, a diad of two sister chromatids from each tetrad side by side but clearly separate. A nuclear membrane forms about each group, and the new nucleus enlarges, the spiral coils of the chromonemata open out and gradually and irregularly lose their chromaticity, while the chromosome membranes become vague.

The second meiotic division opens with reassumption of chromaticity by the threads, which are found in spirals lying side by side, each spiral of two closely associated threads. These spirals shorten, and it becomes apparent that they are paired, each diad pair closely associated at the future fiber-attachment point. The chromosomes straighten out from their spiral form, but twist as they shorten so that the two associated threads—chromonemata—are spirally coiled within each chromosome. After breakdown of the nuclear membrane metaphase and anaphase stages are passed without special feature, except that the chromonemata are gradually uncoiled and come to lie fairly simply in the chromosomes. Spindle-fiber attachment constrictions are present during this division. After organization of the four quartet nuclei the chromosome membranes become vague and the chromonemata appear as parallel threads, or later as rows of granules, near the membrane. Ultimately they disappear and the nucleoli become prominent.

The nuclei of the young pollen grains show double threads as soon as mitotic activity is begun, and these shorten to form the haploid number of split chromosomes, which pass through mitosis in the usual way. Spindle-fiber attachment constrictions are evident, but the satellite seen during the diploid somatic mitoses was not noted. Chromonemata were not seen in the chromosomes either, but these features would be difficult to preserve in fixing through the thick wall of the pollen grain. The nuclei gradually lose their nucleoli, and the generative nucleus elongates, bringing the grain to the condition found at the time of effective pollination.

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DESCRIPTION OF PLATES

PLATE XXVII

FIG. 1. Archesporial nucleus at inception of meiotic activity. × 2060.

FIG. 2. Parallel spiral leptotene threads in very early meiosis. × 2060.

FIG. 3. Synapsis from tangled leptonema (below) to pachynema (above). × 2060.

FIG. 4. Pachynema, approaching the hollow spireme condition, with chromomeres evident. × 1635.

FIG. 5. Pachynema, mostly at closest association of the threads. Portions of spireme, × 2060.

FIG. 6. Pachynema, artifactual representation of chromomeres as large, partly fused droplets on portion of spireme. × 2060.

FIG. 7. Pachynema, on the left, passing into diplonema on the right. × 1635.

FIG. 8. Pachynema, late, passing into diplonema. Portions of spireme, × 2060.

FIG. 9. Strepsinema, early, general view. × 1635.

FIG. 10. Strepsinema, late stage, general view. One element (lower right) beginning to show longitudinal segregation to form a tetrad. × 1635.

FIG. 11. Strepsinema, very late stage, with tetrad nature of some elements clearly recognizable. × 1635.

PLATE XXVIII

FIGS. 1, 2. Strepsinema, late, showing well marked tetrads with constituents well defined. × 2060.

FIG. 3. Strepsinema, late, showing tetrads. At *a* with the chromomeres ill preserved; at *b* in optical section, tetrad of small chromosome pair. × 2060.

FIG. 4. Metaphase (*a*, *b*) or incipient anaphase (*e*) chromosomes of the first meiotic division, polar (*f–h*) and side (*a*, *b*, *e*) views showing differentiation into matrix and chromonemata of various degrees of distinctness. × 2060.

FIG. 5. Anaphase chromosomes showing more obvious chromonemata with differentiation of future fiber-attachment point for second meiotic division, especially clear in the smaller chromosomes (lower right). × 2060.

FIG. 6. Anaphase chromosomes, later stage than figure 5. Note suggestion in lower right-hand specimen of a split in the chromonema. × 2060.

FIG. 7. Telophase chromosomes, early stage, showing increasing diameter of the spiral coils and lessened stainability. × 2060.

FIGS. 8–10. Telophase chromosomes, later stages, showing interrupted chromaticity. Figure 10 *in situ*, others selected elements. × 2060.

PLATE XXIX

FIG. 1. Interkinetic nucleus at inception of second maturation division, the chromatic tracts evident, with some suggestion of continuity. × 2060.

FIG. 2. Prophase of second maturation division, later stage showing spireme elements forming indefinite coils. × 2060.

FIG. 3. Prophase, later stage, with elements clearly spirally coiled. × 2060.

FIG. 4. Prophase, advanced stage, showing the diad split chromosome pairs in polarized relation. Note duplication in the two halves of the figure of the lower right-hand chromosome pair and half of the uppermost one, for orientation purposes. With these duplications the entire complement is illustrated. × 1635.

FIG. 5. Prophase spireme segments, and later chromosomes, to show duplex structure of the former and chromonemata in the latter. *a*, early prophase. *b*, *b*, breakdown of nuclear membrane. *c*, metaphase. *d*, *d*, rather early anaphases. × 2060.

FIG. 6. Anaphase chromosome group, lateral view to show form. × 1635.

FIG. 7. Anaphase chromosome group, very late. Chromonemata, well straightened, are in evidence, the matrix is vacuolated, and a hyaline area is developing about the group of chromosomes preparatory to the formation of the nuclear membrane. × 1635.

FIGS. 8–10. Telophases, progressive stages to show behavior of chromonemata. × 1635.

PLATE XXX

FIG. 1. Prophase, very advanced stage, showing chromosome pairs from the pole opposite that to which the head ends are directed. × 2000.

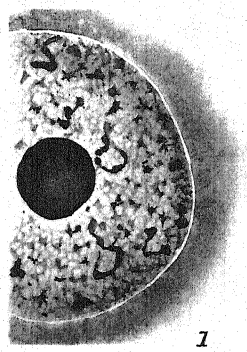
FIGS. 2, 3. Prophases, still more advanced, showing spindle-attachment ends of the split chromosomes. × 2000.

FIG. 4. Prophase, very late, with chromosomes almost straight, and the attachment-ends directed toward one pole. × 2000.

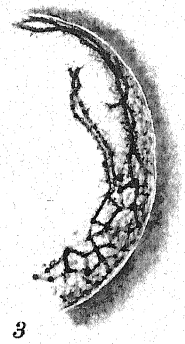
FIG. 5. Prophase, breakdown of the nuclear membrane. × 2000.

FIG. 6. Pollen grain, prophase of the first mitosis, early stage showing short chromatic bits of split spireme. × 2000.

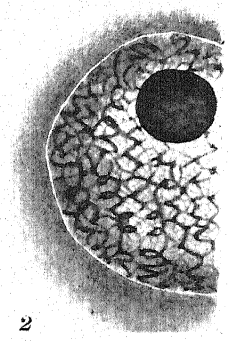
FIGS. 7–12. Pollen grain, prophases of the first mitosis, successive stages showing gradual condensation and shortening of the split chromosomes. × 2000.



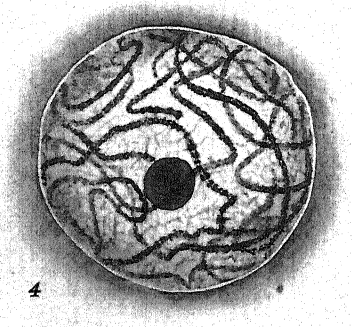
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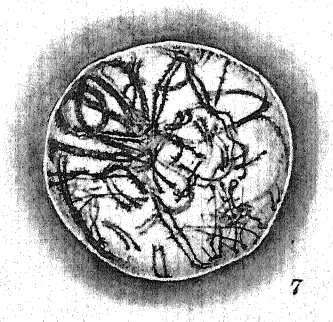
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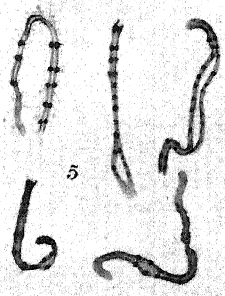
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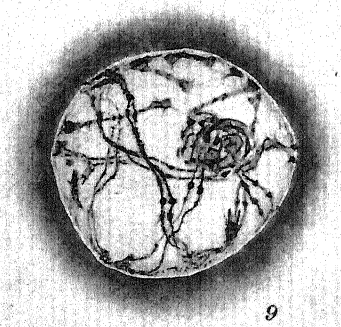
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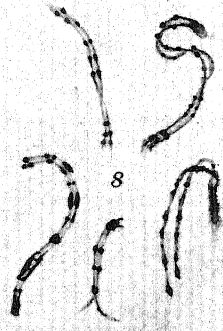
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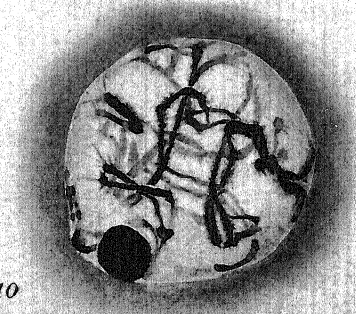
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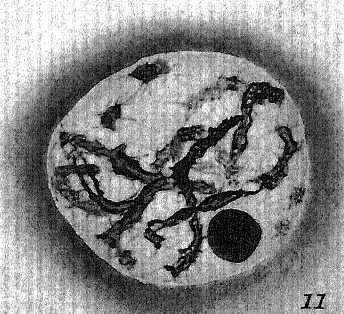
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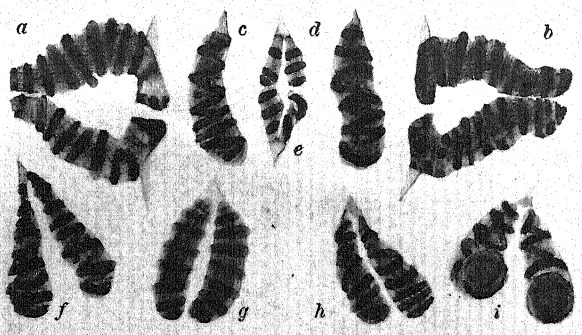
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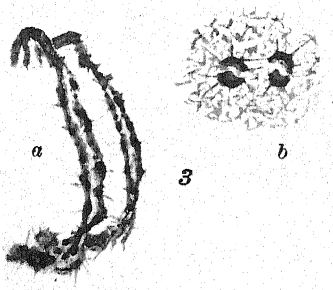
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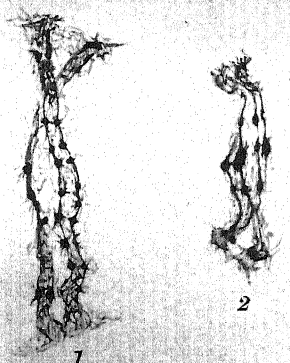
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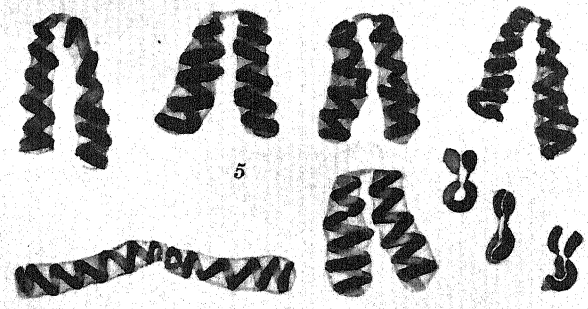


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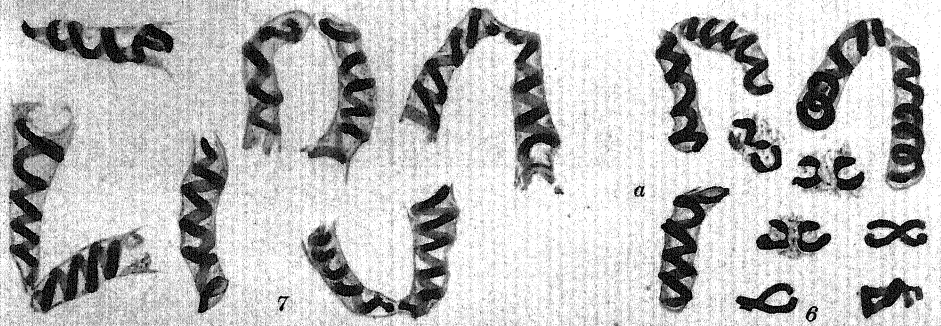


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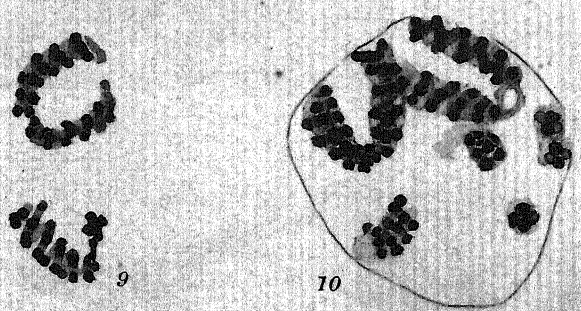


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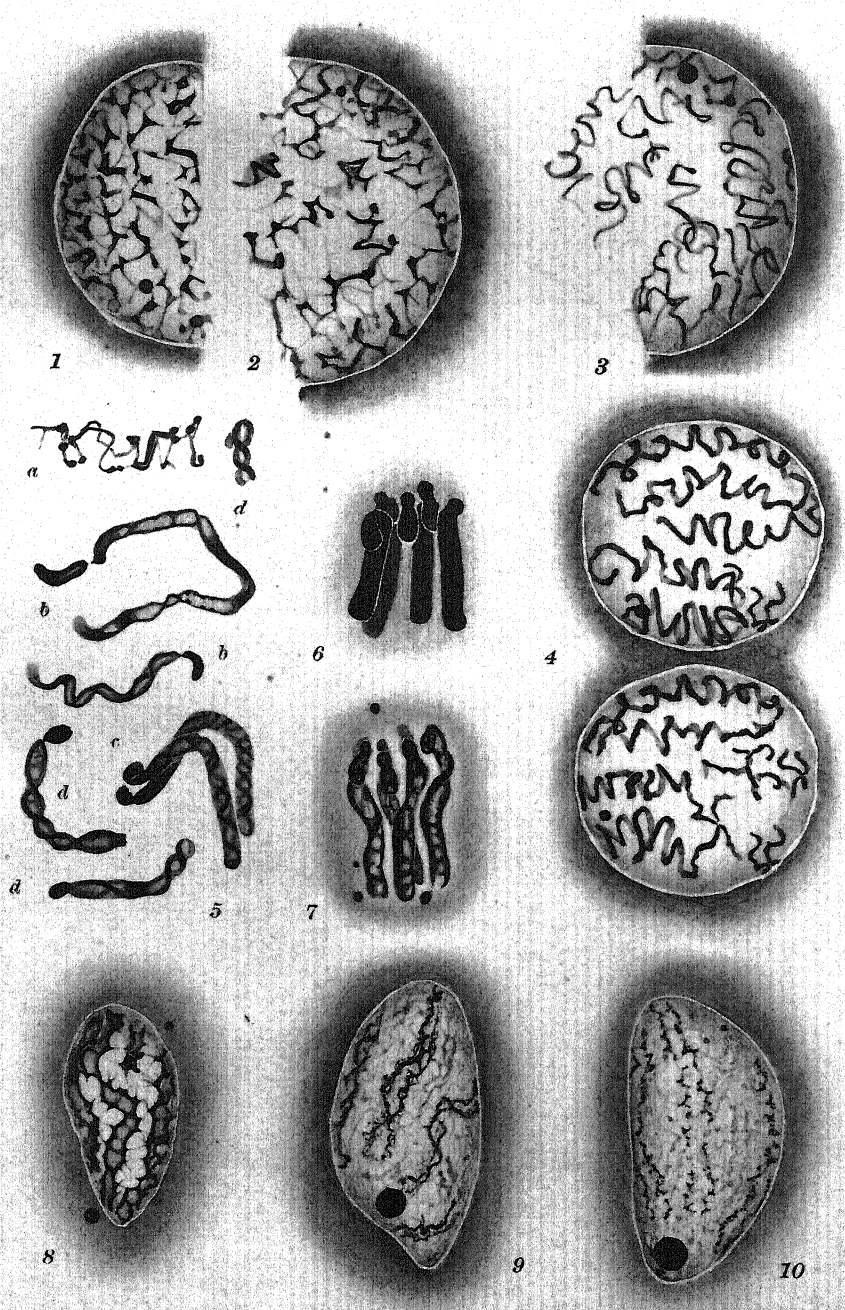


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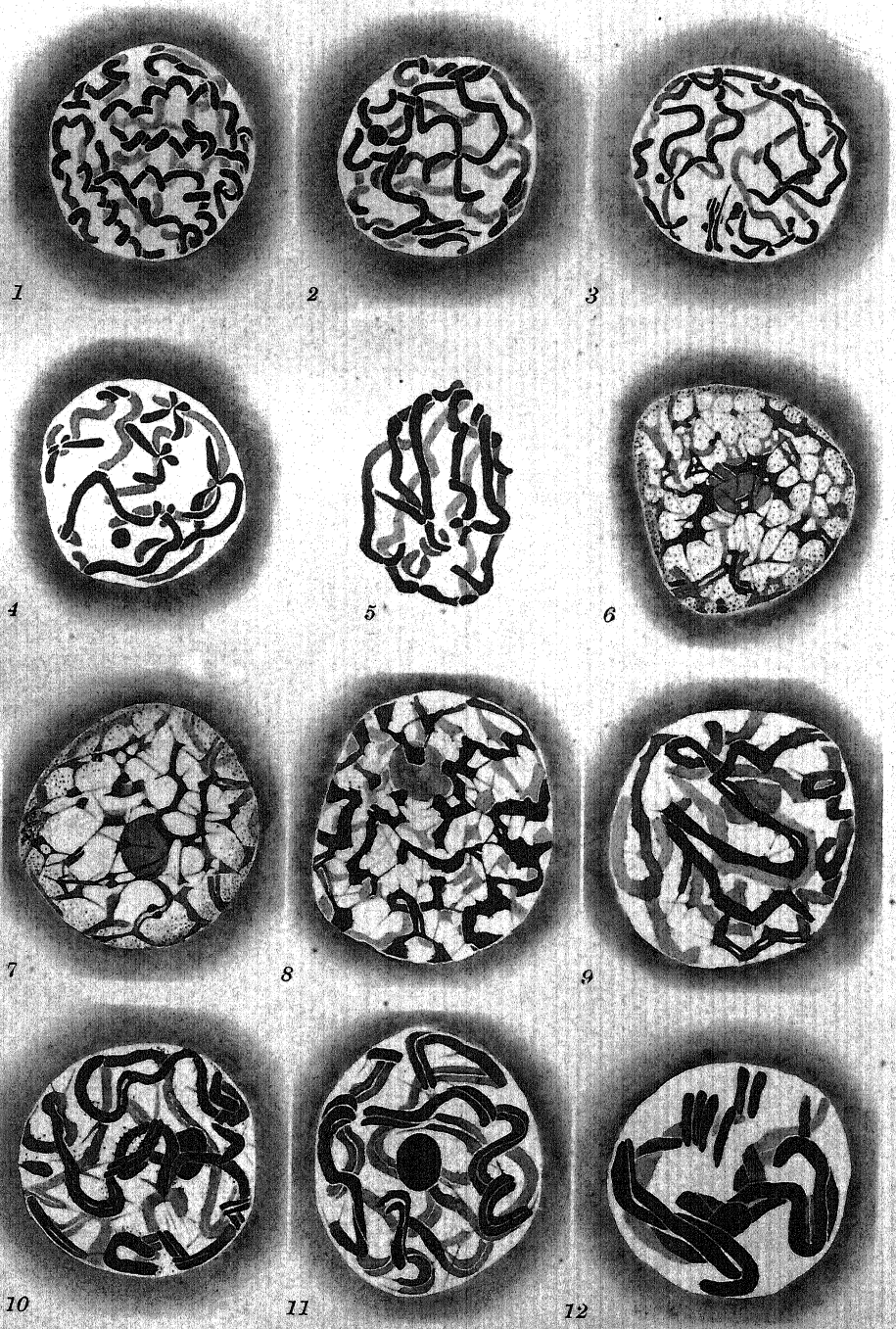
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TAYLOR: GASTERIA



TAYLOR: GASTERIA

A MORPHOLOGICAL STUDY OF THE LEAF NOTCHES OF *BRYOPHYLLUM CALYCINUM*

M. Dorisse Howe

(Received for publication December 1, 1930)

The peculiar behavior of the leaves of *Bryophyllum calycinum* when they are severed from the plant is well known to botanists. Loeb has done extensive work on the physiology of the plant, showing that the growth at the notches of the leaves is a result of conditions brought about by completely severing the leaves from the stem. Loeb, Holm, Sachs, Strasburger and Goebel have referred to "buds" present in the notches or on the surface of leaves of this species. Hofmeister briefly discusses the formation of these "buds." Miss Beals (1) concludes from her work that the "regeneration" in *Bryophyllum* takes place by the division of the phloem cells in the vein; thus the structures formed are endogenous. According to Sachs, in the formation of adventitious or regenerated structures this is usually the case. However, Hofmeister (3) states that the growth occurring in *Bryophyllum* is exogenous, and Goebel that the adventitious structures formed in the leaf notches of *Bryophyllum calycinum* develop from "tissue still embryonic." He further states that there is a "primary parenchyma" in the leaf notches even before the leaves unfold and that the "buds" develop from this parenchyma. In Miss Beals' report the roots have been described as the first structures to appear.

The material studied for this paper was collected from a number of plants at various times during a two-year period of observation. The youngest material which was used was collected from young leaves which were approximately one-sixth of the length of the fully expanded leaves on the same plant. The series of stages studied ranged from stages in the notches of these leaves to stages in the notches from leaves which had been removed from the plant for several days. At the edges of the notches of the youngest leaves there was found a group of small cells with large nuclei and dense cytoplasm. These were distinctly different from the other cells of the leaf and apparently had no connection with the vein. This group of cells gradually multiplied, producing a slight protuberance into the notch. In a few cases the protuberance was slightly lobed. Development beyond this point took place after the leaves were removed from the plant, when definite connections between the vein of the leaf and the developing plantlet were established. Next, leaf primordia were formed, after which the root primordia developed. In all of the material studied, the development of the "bud," *i.e.* the stem primordium, was exogenous, proceeding from a

small group of cells located on the edge of the leaf at the base of the notch. The small cells found in the youngest leaves may correspond to Hofmeister's "Urparenchym," which he believed to be differentiated as the leaf was formed. A period of dormancy seemed to occur, when the leaf was mature, in which the "bud" remained until stimulated to further growth by the removal of the leaf from the plant or by some other factor. At about this time two root primordia were formed endogenously somewhere near the vein and pushed their way out through the leaf tissue. Their exact origin was not determined.

In regard to the behavior of the roots after the primordia are formed this study confirms the report of Miss Beals that the roots push out through the leaf. Confirmation of her statement that the roots are formed from the primary phloem is at present impossible. Miss Beals states that "regeneration" in *Bryophyllum calycinum* starts with the division of the phloem cells in the vein, and seems to conclude that the first structures formed are roots, developed soon after the leaves are removed from the plant. The present study shows that the stem primordium is the first structure to develop and that this development begins in the very young leaves. Plate XXXI, figure 7, shows a section which closely resembles that in Miss Beals' paper. In this section the two roots have begun to push their way through the leaf tissue. Figure 8 shows another section in the same series of sections, in which the stem tip is growing into the notch. In every case in which the root primordia were present the stem primordium was well developed, and in some cases the stem primordium was present when the root primordia had not yet developed. While Miss Beals may be right in her statement that the roots develop from the phloem, it is the belief of the writer that she is not justified in maintaining that the root development is the beginning of growth in the notch, or that all of the growth from the notch is endogenous.

Loeb states (5, 6) that growth will not normally occur in the notches of leaves of *Bryophyllum calycinum* while the leaves are still attached to the plant, and similar conclusions have been published by Strasburger, Holm (4), and Goebel. Miss Braun (2), however, found that plantlets sometimes developed on leaves which were still attached to the plant. She stated that the plant in which this was observed was under normal conditions for a house plant, and that it seemed to be perfectly healthy. The fact that the plant was not erect was believed by Loeb to result in an inhibition of the flow of substances, owing to the constriction at the bend in the stem. He maintained that because of this inhibition the notches developed, and that since the flow of substances was not normal the plant was a "sick" plant (7). Dr. Ernest Reed (8) has found a number of examples similar to that of Miss Braun in the greenhouse of the University of Michigan. Plate XXXI, figure 9, shows one of the plants studied by the writer in the Syracuse University greenhouse, the leaves of which produced plantlets while still

attached to the parent plant. These plants were all erect, and appeared to be perfectly healthy as evidenced by a good color of the foliage and a vigorous growth of the terminal shoots. On two of the plants the leaves of the plantlets had also produced a growth in their notches, giving two successive generations of adventitious growth all attached to the parent plant. All of these plants were under normal greenhouse conditions. Some were growing in pots and some in the soil on the greenhouse benches. In all of the cases considered the leaves showing adventitious growth were free from contact with the soil. These observations would seem to bear out the conclusions of Miss Braun that Loeb's results are not substantiated by the behavior of such plants as these.

Summary

1. The growth in the notches of the leaves of *Bryophyllum calycinum* is exogenous.

2. The growth proceeds from a meristematic group of cells which is present in the very young leaves.

3. The growth, which may be termed a "bud," is definitely organized when the leaf is mature.

4. The roots are formed after the formation of the stem primordium and are endogenous.

5. Growth of the leaf notches producing well developed plantlets is possible while the leaf is still attached to the plant, and while the parent plant is, as nearly as can be observed, in a healthy condition.

The writer wishes to express her appreciation for aid received from Dr. Paul J. Sedgwick in the preparation of this paper. Thanks are also due to Dr. Ernest Reed for his helpful suggestions.

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EXPLANATION OF PLATE XXXI

Magnifications: FIGS. 1, 2, 4, × 400; FIG. 3, × 60; FIGS. 5, 6, 7, 8, × 85; FIG. 9, × ¼.

Figures 1–5 are from leaves removed from plant just prior to killing.

FIG. 1. Notch from youngest leaf examined, showing group of small cells at edge of leaf. Section parallel to leaf surface.

FIGS. 2, 3, 4. Older stages of "buds." Sections parallel to leaf surface. Fig. 4 is a detailed view of "bud" in figure 3.

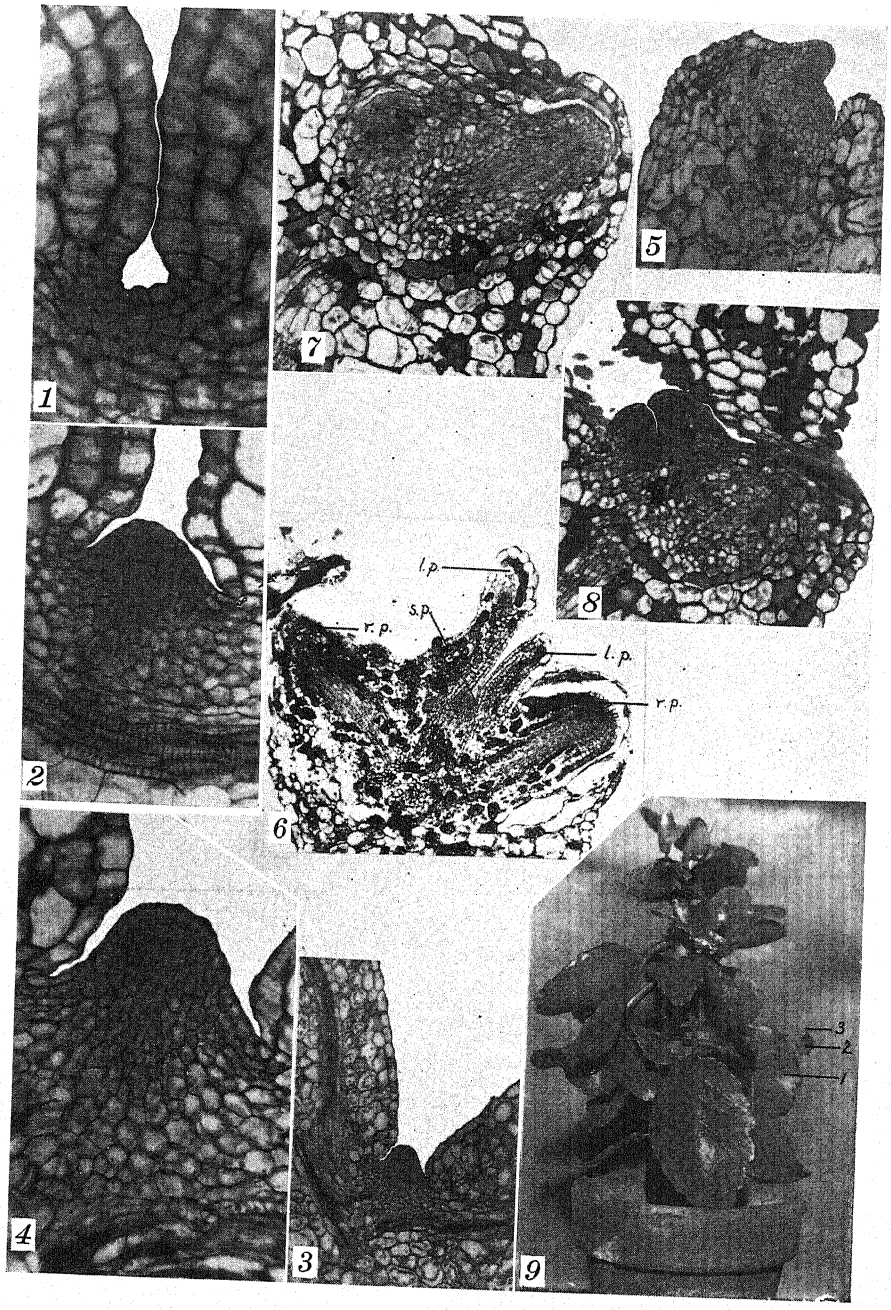
FIG. 5. Section of "bud" at right angles to leaf surface, about same stage of development as figure 4. This is the stage of development of the "bud" when the leaf is mature.

FIG. 6. Section of "bud" showing two root primordia, *r.p.*, two leaf primordia, *l.p.*, and a stem primordium, *s.p.* Cut at right angles to leaf surface. Leaf removed from plant several days.

FIG. 7. Section through "bud" cut parallel to leaf surface, showing two root primordia. Similar to drawing of Miss Beals.

FIG. 8. Section through same "bud" as figure 7, showing two leaf primordia.

FIG. 9. Whole plant showing three generations of plants (1, 2, 3) all attached to mother plant.



HOWE: BRYOPHYLLUM

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THE ROOT SYSTEMS OF TREES GROWING IN SPHAGNUM

GEORGE B. RIGG AND E. S. HARRAR

(Received for publication December 20, 1930)

This paper reports the character and distribution of roots of coniferous trees growing in bogs whose surface layer is composed of sphagnum peat. The investigation was largely prompted by the observation that wind-falls are extremely rare in bogs, although they are common on both glacial till and muck soils in the same region. Raw peat constitutes a very loose substratum as compared with the other two substrata observed. Thus the character and distribution of the anchorage which enables large and mature trees scattered in the bogs to withstand heavy wind seemed worthy of investigation.

Four bogs, Ronald, Esperance, Schrieber Lake, and Evans Creek, were examined. All are within a few miles of Seattle, Washington. The surface layer of these bogs is from one to three feet deep and is composed of dead sphagnum which has not become disintegrated. In some portions there is living sphagnum at the surface. Immediately below this surface layer of dead sphagnum there is either a layer of mud one foot or more in thickness or a much thicker layer of sedge peat. Even when a layer of mud is present there is generally a layer of sedge peat beneath it. The total depth of organic matter in these bogs varies from six to thirty feet (Rigg, 8).

An abundant flora of low shrubs characterizes these bogs (Rigg, 8). Two of them, *Ledum groenlandicum* Oeder and *Kalmia polifolia* Wang, give the aspect to the bogs. *Vaccinium oxycoccus* L., *Drosera rotundifolia* L., *Eriophorum chamissonis* Mey., and two sedges (*Carex* spp.) are the other species which form a part of the vegetative floor of these areas. In their mature stage they are invaded by trees. Although the encroaching species are largely coniferous, the western birch (*Betula occidentalis* Hooker) is common when the species is a constituent of the near-by forest.

Six conifers: *Tsuga heterophylla* (Raf.) Sargent, *Pinus monticola* D. Don., *Pinus contorta* Loudon, *Thuja plicata* D. Don., *Picea sitchensis* (Bongard) Carriere, and *Pseudotsuga taxifolia* (LaMarck) Britton; are found in these bogs. The first species listed above is the most common and the last is

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never abundant. The root systems of these six conifers in bogs were the subjects of this study. With the exception of *Picea sitchensis* which in the vicinity of Seattle and environs is found only in bogs and swamps, control studies were made of these root systems, growing under normal conditions, mainly on glacial till.

Vertical Penetration of Roots

A careful examination of the roots of the above mentioned conifers showed a striking similarity in the distribution of their subterranean structures. As a group their lateral roots were observed to be only a few inches (rarely more than two feet) below the surface of the bog, wholly embedded in the raw sphagnum layer. None of them except *Pinus contorta* showed tap roots. In no instance were the living roots found in the mud or the layer of sedge peat. A characteristic undulation of these lateral roots in the vertical plane is apparent. This phenomenon is probably accounted for through the rise and fall of the growing tips as they course their way through the sphagnum immediately above the fluctuating water table. Sapling root systems wherein little thickening had occurred best depicted this undulation.

Length of Lateral Roots

Our observations in all cases indicate excessive elongation of roots growing in peat soils. The unusual length of the roots in proportion to the height of the tree occurs with seeming regularity in all species growing in sphagnum. Below are listed the relative lengths of roots and stems of trees growing in sphagnum and in glacial till.

Trees growing in peat:

Species	Height	Maximum Length of Root
Pinus contorta	5 ft.	13 ft.
Pinus contorta	8 "	23 "
Tsuga heterophylla	4 "	16 "
Tsuga heterophylla	20 "	33 "
Thuja plicata	30 "	21 "[1]
Pinus monticola	12 "	17 "
Pinus monticola	60 "	47 "
Picea sitchensis	12 "	11 "

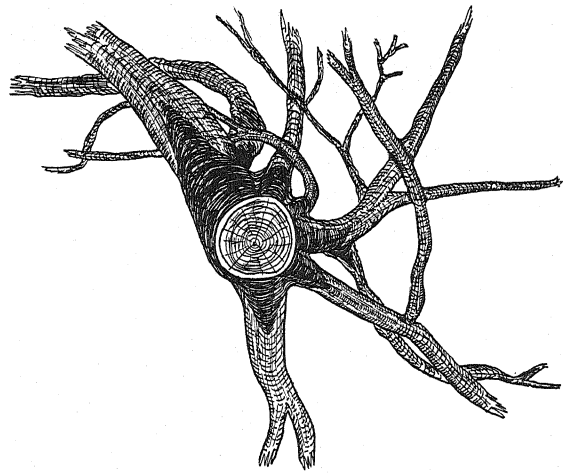
Trees growing on glacial till:

Tsuga heterophylla	8 "	9 "
Tsuga heterophylla	30 "	17 "
Pseudotsuga taxifolia	40 "	18 "
Pinus monticola	15 "	11 "
Pinus monticola	35 "	23 "
Pinus contorta	8 "	4 "
Pinus contorta	12 "	7 "

[1] Root elongation to south limited by presence of a lake in the bog.

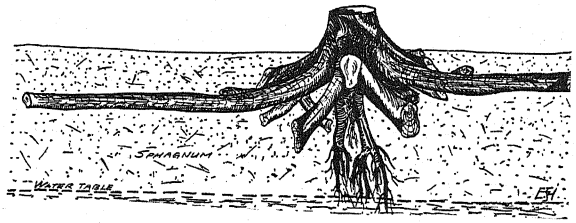
According to Büsgen and Münch (2), "The wealth of branching and the whole appearance of the root system are governed in a striking manner by the nature of the soil into which they penetrate." They further indicate that greater root systems are developed in sandy and humus soils than in harder soils, and that mechanical obstructions rather than conditions of nutrition seem to retard root elongation. Our observations are in accordance with these generalizations.

The root systems of *Pinus contorta*, *Pinus monticola* (text fig. 1), and



TEXT FIG. 1. Diagrammatic sketch of a root system of white pine (*Pinus monticola*) depicting characteristic branching and root fusions in sphagnum.

Tsuga heterophylla are sparingly branched, but abundant branching is the general rule in the other four species in sphagnum, glacial till, and muck, and there appears to be no correlation between root branching in any one species and the character of the soil in which it grows.



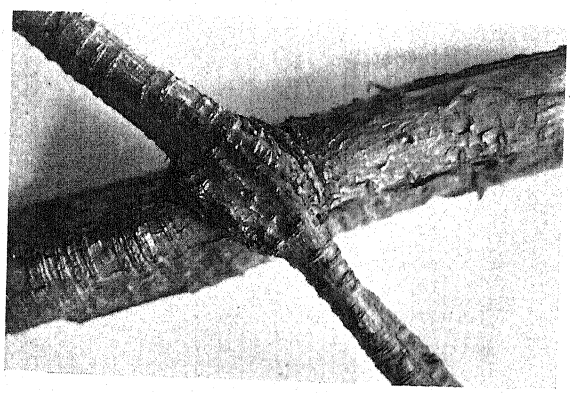
TEXT FIG. 2. Plan illustrating the radial and vertical distribution of the roots of lodge pole pine (*Pinus contorta*) in sphagnum.

The root systems of *Pinus contorta* are distinctive in that, in addition to exhibiting a well-defined lateral anchorage, a peculiar tap root has also been developed. This structure (text fig. 2) is short and stout and is profusely branched. In the older trees two or three of these queer, excessively

branched structures are to be seen. The vertical penetration of these organs is, however, only a few inches below that of their lateral associates. Probable factors in the preclusion of further growth in this direction are lack of oxygen, abundance of carbon dioxid (Rigg, Thompson, Lorah, and Williams, 9) and high acidity (Thompson, Lorah, and Rigg, 10). The tap root is well developed in lodge pole pine in this region when it grows in glacial till.

Root Fusion

The root systems of all the larger trees examined in peaty soils exhibited some root fusion within the system, but no fusion between systems was found. It seems probable that the swaying of the trees in the wind causes the bark of the roots to be worn off where they come into contact in the complicated, ramifying growth that characterizes the root systems of trees growing in sphagnum, and that the root fusions result when meristematic tissues are thus brought into contact. It is well know that root fusions between systems do occur in Douglas fir (*Pseudotsuga taxifolia*) growing in glacial till, but fusion within the system has not been investigated so far as the writers know. Fusions between the stems of the red alder (*Alnus oregona*) where they have been rubbed together by swaying in the wind are well known in the Seattle region. It seems probable that the root fusions within the system in trees growing in sphagnum gives rise to increased



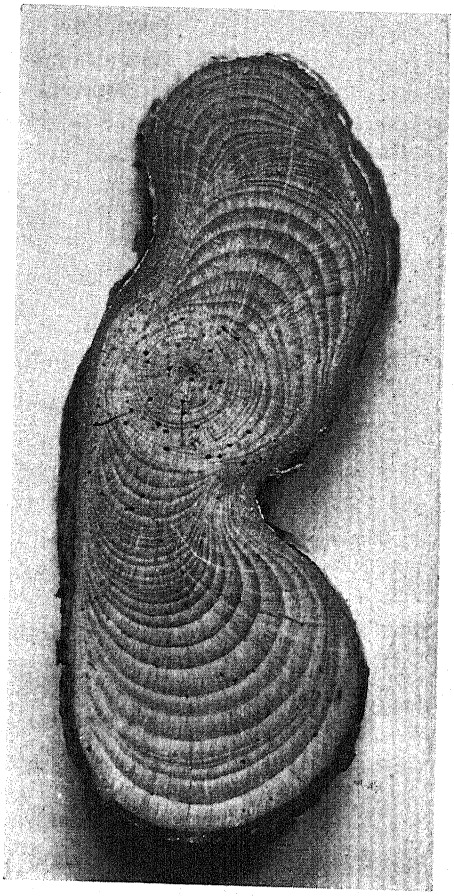
Text Fig. 3. A typical root fusion found in white pine roots.

rigidity in the anchorage and thus increases the mechanical efficiency of these organs. Text figure 3 depicts a fusion typical of those found in the bogs investigated.

Eccentricity in Roots

Probably the most striking discovery made in this work is the strange shapes of the old roots. In cross section, young roots are circular or nearly so. On the other hand, the older ones are found to be very deep in proportion to their width (text fig. 4). Though the eccentricity of growth was

on both the top and the bottom of the root, there was much more growth on the bottom than on the top. The transverse sectional shapes of these roots conforms to three general types, namely; the oval, the T-girder, and the I-beam. The I-beam or sometimes nearly rectangular structures were most abundantly found. An examination of tree roots of the same species



TEXT FIG. 4. Transverse section of I-beam-like root of white pine. Similar structures were observed in all species growing in sphagnum that were examined.

growing in the firmer glacial till and muck soil depicted a striking contrast with those growing in the sphagnum peat. Although some of the older roots in glacial till and muck approached the rectangular shape near the base of the tree, the more nearly circular form was by far the most common type observed.

The increase in mechanical efficiency with the increase in the vertical axis of the transverse section as compared with the horizontal axis is

apparent. It is known that the strength of beams of varying transverse sectional shapes approximates the square of the vertical dimension when the horizontal dimension remains the same.

A rather special case of eccentric growth in roots in sphagnum was observed by the writers in the roots of the western red cedar (*Thuja plicata*). Where these roots have undulated in growth while young, excessive growth has later occurred on the concave side of each curve so that the root is much larger at the curves than in the comparatively straight portions between them. In a characteristic section of one of these thickened regions which was studied in detail, the eccentricity had begun as early as the third year of its life. On the side showing the greater growth, 28 annual rings were counted, constituting a total increment of $2\frac{3}{4}$ inches, while on the other side the total increment was only three-eighths of an inch and the rings were so merged that only 20 could be counted.

Eccentricities of growth in stems and roots have long been known. In 1803 Knight (5) arranged apple saplings by a mechanical device so that the wind could sway them only in the arc of a circle, and found that at the end of a year the diameter of the stems in the direction in which they were most exercised by the wind exceeded that in the opposite direction in the proportion of 13 to 11. He also found that when a rigid support was placed around a young tree some distance above the soil so that the wind could sway the trunk above the support, the greatest growth in thickness occurred just above the support. He concluded that the greatest growth takes place where there is the greatest movement.

Burns (1) found that pine trees bent over and fastened in that position for several years produced eccentric growth on the lower side. He considers this a morphogenic response to gravity.

Excessive growth of tree trunks at the point where weight is supported has often been noted. Peirce (6) has called attention to the large brackets formed under heavy lateral branches in redwood trees, and the writers have often observed that where the upper portion of a red cedar tree has been broken off in logging operations and a branch growing nearly horizontally has remained living, a supporting bracket is formed under the branch as it thus makes unusual increase in diameter. Kienholz (4) has cited the literature of eccentric growth in stems.

Both Hartig (3) and Rasdorsky (7) have experimentally demonstrated that swaying stems bring about eccentricity of root development, and have demonstrated that the greatest thickening occurs in the plane of the bending. Büsgen and Münch (2) have found that the roots of Norway spruce growing in peaty soils show eccentric annual increments. The present study indicates that this phenomenon is extended to all of the local species examined growing in sphagnum peat.

SUMMARY

1. The depth of tree root penetration in peat soils is controlled mainly by the height of the water table, though in some cases lack of oxygen, abundance of carbon dioxid, and high acidity may be factors.

2. The root systems of trees growing in sphagnum are far more spreading than those of the same species growing in firmer soils and mat-like pedestals often develop.

3. Excessive root fusion often occurs in sphagnum peat.

4. Eccentricity of root growth and enlargement is characteristic of tree roots growing in sphagnum peat.

5. The combined effect of root fusion and elongation coupled with root eccentricity increases the mechanical efficiency of the anchorage of the trees growing in sphagnum peat and these three phenomena are largely responsible for the lack of wind-falls in the bogs of the north Pacific coast.

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CYTO-GENETIC INVESTIGATIONS OF A HAPLOID TOMATO AND ITS DIPLOID AND TETRAPLOID PROGENY[1]

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(Received for publication December 26, 1930)

INTRODUCTION

During the past five years a haploid tomato (*Lycopersicum esculentum*) has been under observation both from the cytological and the genetical viewpoints. Since its appearance as a single individual in 1926, the haploid has been continued asexually by cuttings. Diploid offspring from it have been derived both sexually and asexually. Tetraploid progenies have also been produced.

The original haploid with twelve univalent chromosomes arose spontaneously in an F_2 generation from a normal varietal cross involving neither sterility nor any other abnormal conditions (Lindstrom, 1929). Presumably it traced to the parthenogenetic development of an egg cell, a very rare phenomenon in the tomato.

Vegetatively the haploid is normal in appearance although noticeably reduced in all parts. Comparative measurements of stem, leaves and flowers prove it to be approximately 70 percent of the size of a typical diploid. The staminate and pistillate portions, although smaller, are essentially normal, showing that, as far as these features of sex are concerned, the haploid quota of chromosomes is wholly sufficient for the development of an hermaphroditic plant. The sexual cells themselves are highly sterile, both micro- and megaspores showing a high degree of abortion as the result of the absence of a normal reduction division. Consequently the haploid is almost completely sterile.

The literature on haploidy in plants is beginning to assume considerable proportions. A very recent review by Gates (1930) which covers the field thoroughly would seem to render unnecessary any further compilation here. Kuhn (1930) has also lately reviewed the literature on haploidy.

Before considering the cytological aspects of the problem, it seems advisable to discuss several general points as a basis for the study of the haploid and the diploid and tetraploid forms derived from it.

PRODUCTION OF DIPLOID AND TETRAPLOID FORMS FROM THE HAPLOID

There are two methods of obtaining diploids from the haploid. First, the sexual method, is the self-fertilization of the almost sterile haploid. Pollen counts show 98 percent or more of shriveled or abortive grains.

[1] Paper No. 36, Department of Genetics, Iowa State College.

Much the same situation exists with the megaspores. Attempts at self-pollination end in failure over 95 percent of the time. A few fruits with 1–3 fertile seeds have been obtained. These produce fully fertile diploids. At the present time eight lines of diploids from this sexual origin have been grown and tested genetically with every indication of their being completely homozygous as well as completely fertile.

The second, or asexual, method of producing diploids from the haploid, is accomplished by decapitating young haploid plants and permitting sprouts to develop from adventitious buds in the callus tissue formed by such decapitation. All axillary buds are carefully removed. Modifications of Jorgensen's (1928) method have proven very successful. Various treatments of the callus to induce rapid and healthy growth of the adventitious buds were tested. These included moistened peat, moss, and cotton held in place on the callus, as well as adhesive tape and petrolatum. The latter treatment gave the best results. To stimulate cellular changes in the callus, it was inoculated with *Bacterium tumefaciens*, and also treated with various dilute solutions of chloral hydrate, ethylene chlorhydrin, and ether, but with no success. Large calluses were produced by *B. tumefaciens* but they proved to be too woody for good regeneration of sprouts.

Using petrolatum (or vaseline) over the young, cut stem keeps the wound tissue fresh; and within two weeks adventitious buds may be seen on the callus. These eventually develop into sprouts which are cut off and rooted as cuttings. Ninety-four such sprouts from the haploid have been grown to maturity. Of these, thirty proved to be completely fertile, and had the phenotypic appearance of diploids. Cytological tests, to be discussed later, demonstrated that they were diploid. The remaining sprouts when mature were typical haploids. Thus more than thirty percent of the adventitious buds in the haploid callus of this experiment had in some manner become diploid with this treatment.

Although the haploid has been carried on by cuttings over a period of five years, not a single one of the tested 600 or more cuttings has deviated from the original type phenotypically. Earlier work had shown a few diploid cells in the root tips of the haploid. Had this occurred in aërial tissue, involving the growing meristem, the change to the diploid would have become manifest in a return to complete fertility, a characteristic that could not easily have been overlooked in the tomato. Why there is diploid tissue in the roots and not in aërial tissue is a point of great interest for which there is no apparent answer. Likewise, there is as yet no clue for the very high percentage of diploid cells in the callus of decapitated haploid plants.

From these regenerated diploid plants, which are completely homozygous, tetraploid forms have been produced by this same callus method. Three hundred and nine sprouts from decapitated diploids have been grown and tested for tetraploidy by phenotypic appearance and pollen obser-

vations. Of these, 109 were unquestionably tetraploids with typically larger stems, leaves, and flowers. Some of these were investigated cytologically and proven to be tetraploids.

Cytological Methods

The haploid and regenerated diploid buds to be used for cytological preparations were removed from the plants at various hours of the day. Those taken at about 8 A.M. and 2:30 P.M. during the winter months were found to provide the most and best figures. In order to insure rapid penetration of the material, the calyx and the corolla of each bud were removed, and the bottle of killing solution containing the buds was put under a suction pump for about ten minutes.

Of several killing solutions tried, three were found to give very satisfactory results: Bouin's solution, Allen's modification of Bouin's solution, and acetic alcohol consisting of three volumes of methyl alcohol to one volume of glacial acetic acid. Some shrinkage of the cytoplasm occurred, but in almost all of the stages studied this condition was an advantage.

The callus tissue from the decapitated young plant stems to be studied cytologically was split longitudinally in order to afford a greater surface for penetration, and the air was removed from the tissue with an aspirator. Bouin's solution was used as the fixing agent.

The buds and the callus were imbedded in paraffin by the usual methods. Iron-alum haematoxylin was used as a stain.

Cytology of the Haploid Tomato

The microsporocytes of the haploid tomato range in diameter from 11.2 μ to 12.8 μ. The diameter of the nuclear cavity is from 8 μ to 9 μ, and each metaphase chromosome is approximately a single micron in length. Since the subject with which this work is concerned is of such small dimensions, emphasis has necessarily been placed upon the more obvious metaphase phenomena, rather than upon those of the prophase.

A microsporocyte in an early prophase stage has a large, well-defined nucleus surrounded by a distinct nuclear membrane. The early spireme is the usual fine, chromatic filament, along which are distributed at irregular intervals small, deeply staining granules. A progressive shortening and thickening of the entire spireme takes place until the chromatic portions appear as a more or less continuous, convoluted, coarse spireme (Pl. XXXII, fig. 1). No doubleness was noted in the spireme at any of the stages studied. Finally, fragmentation occurs and twelve distinct, univalent chromosomes are formed.

There is a massing of the univalent chromosomes following their formation. The entire group stains very deeply and the individual chromosomes are indistinguishable. The individual chromosomes do not become distinct immediately after emerging from this massing stage, as there is a

'stringing-out' of the chromatic material. Several of the chromosomes may be held together by short strands, or two or more may be connected by long, thin threads (fig. 2).

Spindle fibers now appear and a bipolar spindle, or, more rarely, a multipolar figure is formed. The chromosomes in either case do not form a distinct metaphase plate, and two or more chromosomes may remain attached for a time (fig. 3).

In the case of the formation of a bipolar spindle, the distribution of the chromosomes during the anaphase differs widely in the individual pollen mother cells. Conclusions can be made only from observations on figures which have the total number of chromosomes visible on the spindle. Since no metaphase plate is formed, there is no basis for an orderly procedure of the chromosomes to each pole. An equal distribution may take place so that there are six chromosomes at each pole (fig. 4), but more commonly combinations of 5 and 7, 4 and 8, and 9 and 3, are observed (figs. 5, 6, 7). Microsporocytes having 10 and 2, and 11 and 1 chromosomes at opposite poles have also been noted (figs. 8, 9). However, it is relatively difficult to ascertain the exact numbers in such cases due to the massing of the large number of chromosomes at one pole. In view of these observations, one may expect to find rare instances in which the entire group of twelve chromosomes has gone to a single pole. It is difficult, however, to determine whether such an anaphase distribution has taken place in a bipolar spindle, or whether there has been no spindle formation whatever.

Multipolar spindles are the exception rather than the rule, although large numbers of them have been observed (fig. 10). The chromosomes are scattered over the entire multipolar figure in an irregular fashion, and one or two may even be extruded into the cytoplasm. The distribution of the chromosomes is necessarily a chaotic one, and irregular numbers are drawn to the various poles of the figure.

Interkinesis, with the formation of a nuclear membrane about each of the daughter nuclei, does not occur in the microsporocytes of the haploid tomato. The chromosomes gather into more or less distinct groups followed immediately by the formation of bipolar figures (fig. 11). Since more than twelve chromosomes are present in the entire pollen mother cell at this stage, it is evident that some of the chromosomes have split. No metaphase plate is formed, however, and the distribution of the univalent and halves of univalent chromosomes into groups is at random.

As a result, the number of groups and the number of chromosomes in each group is irregular (fig. 12). Small nuclei having only one or two granules are scattered throughout the cytoplasm and frequently such isolated granules do not appear to have distinct nuclear membranes. Large nuclei containing as many as thirteen chromosomes occur. Such a variation in size and contents of the nuclei suggests that the resulting pollen grains may not have equal functional viability.

It is uncertain whether figures 11 and 12 are the results of the second division as described above, or the products of multipolar figures of the first division. All these stages may be present in a single anther.

The results of cytokinesis are not uniform, there being produced, instead of the usual tetrad of four equal cells, groups of two to six cells of various shapes and sizes (Pl. XXXIII, figs. 13, 14). Diads are not uncommon. Their two cells may be apparently the same in size and nuclear composition, or one cell and its nucleus may be much larger than the other (fig. 13). The formation of diads may be due to the failure of a clean-cut separation of the chromosomes in the second division; to the disintegration of small nuclei, leaving only two large nuclei which may function; or to non-reduction as in *Datura*.

Pollen grains, as they separate, present a far from normal appearance (fig. 15). Many are small and have irregularly shaped nuclei which contain one or two granules. Some seem to be of the normal size, but their nuclei may also have only a few chromatin bodies; and still others may have as many as three nuclei, two of which are small and abnormal. It is probable that the occasional functional microspores are those which have received the necessary twelve chromosomes. Since there is no obvious attempt at chromosome pairing in microsporogenesis, we can be reasonably certain that twelve is the basic, univalent number of chromosomes in the tomato.

Cytology of the Regenerated Diploid Tomato

Two kinds of plants develop from the adventitious buds arising from the callus of the decapitated haploid. Some of the plants derived from these buds resemble vegetatively the haploid, and have the same type of pollen sterility. Others, however, develop into plants differing vegetatively from the haploid, and having good pollen. On the basis of their genetic behavior, and their phenotypic identity with standard diploids, the latter plants are believed to be regenerated diploid plants.

Observations made during the present investigation show that the microsporocytes of these regenerated diploids are noticeably larger than those of the haploid. In the prophase stages they have a diameter of 16 μ to 19.2 μ (haploid averages 12 μ) with a nucleus of about 9.5 μ in diameter.

The nucleus is enclosed in a well-defined nuclear membrane and contains a large, deeply staining nucleolus. The prophase spireme is an irregularly-staining, chromatic filament with granules of uncertain number scattered on its length at irregular intervals. The spireme shortens and thickens until it becomes highly convoluted. Following this stage a fragmentation of the spireme occurs, and the thread-like fragments were observed to be paired and twined about each other, forming a typical strepsinema figure. Contraction continues in each strand until twelve clear-cut, deeply-staining pairs of chromosomes are present. This is a typical diakinesis stage (figs. 16, 17). These pairs of chromosomes gather on the equatorial plate

of the spindle and a typical reduction division takes place, twelve chromosomes going to each pole (fig. 18). This behavior is similar to that in standard diploid tomatoes as described by Lesley (1926).

Cytology of the Haploid Callus

Having verified the fact that these regenerated plants from the haploid are true diploids, it becomes necessary to investigate the cytology of the regeneration process. Between one and three weeks after decapitation of the young haploid plants small protuberances can be noted on the cut surface or callus. In time these form a more or less irregular ring, and the newly made mass of callus tissue then folds inward to cover the entire wounded surface. The vascular bundles of the tomato are bicollateral. The callus apparently arises by the proliferation of the cells of the internal phloem.

Examination of the callus tissue on haploid plants, killed two weeks after decapitation, shows numerous mitotic figures, the majority of which are obviously those of haploid cells (Pl. XXXIV, figs. 21, 22). The telophase figures are highly variable.

In some cells such as shown in figures 24 and 25 the nuclei are in the same late telophase, but the spindle fibers have disappeared, and the two nuclei lie in close proximity in a small body of cytoplasm. Such binucleate cells are relatively common in the callus tissue. Figure 26 shows a nucleus which strongly suggests a recent union of two nuclei, and figure 23 is a polar view of a late metaphase stage in a cell having the doubled number of chromosomes. Many giant nuclei are to be seen in the callus (fig. 28). The cell shown in figure 27 contains conjugate spindles.

From such observations it seems highly probable that chromosome doubling has occurred by nuclear fusion before cell-wall formation had taken place. By this method, then, there is the change from the haploid to the diploid condition.

The exact details of this doubling are not clear. The question arises as to which of the following suggested alternatives brings about doubling:

(1) a failure of a complete anaphase separation with the formation of a single nuclear membrane about the entire diploid group of chromosomes;

(2) the formation of a membrane about each daughter nucleus and the subsequent dissolution of the membranes along the plane of contact as the two nuclei come to lie in close proximity in a single mass of cytoplasm;

(3) the union of the nuclear contents of two nuclei at the prophase stage of the next division; or,

(4) the union of two spindles of a binucleate cell to form a giant spindle, as Jorgensen (1928) suggests in his scheme of 'endo-duplication.'

Evidence of the first possibility is difficult to obtain because it is not clear from observations of the mitotic phenomena whether a given figure has reached the anaphase stage, or whether the anaphase separation has

failed to take place. Figure 26 strongly suggests that there has been a breaking down of the nuclear membranes along the line of contact of the sister nuclei, thus conforming to the idea of the second alternative. The third suggestion has not been observed in the present investigation, but it is altogether possible that such a procedure may occur. Conjugate spindles have been noted in the same cell (fig. 27). However, there was no positive evidence of a coalescing to form a giant spindle, as the fourth possibility suggests.

In view of the general abnormal and irregular character of the nuclear processes in the callus a doubling of the chromosome number may well be accomplished at various points in the mitotic cycle, or by more than one method. Why such a doubling never seems to occur in ordinary somatic tissue (some 600 cuttings of the haploid have never given a diploid plant) is perhaps strange, especially when doubling is known to occur in the root tips of the haploid with reasonable frequency. The high frequency of doubling in callus tissue may be a response to wounding which induces such a rapid mitosis that normal procedure in cell division is upset and the cell wall remains unformed. It is also conceivable that the use of petrolatum on the callus with a reduction in oxygen availability might disturb cell division sufficiently to cause the high frequency of doubling.

Genetic Behavior of Diploids from the Haploid

Cytological observations having demonstrated that the diploids derived from the haploid possess twelve pairs of chromosomes, it is highly probable that these twelve chromosomes have identical homologues. Accordingly these diploids should be completely homozygous; and this should be tested genetically.

The phenotype of the original haploid led us to predict its genotype as *RYdP* (red flesh, yellow skin, dwarf growth habit, and smooth—non-pubescent—fruit). The diploid form proved to be completely homozygous for red flesh (*RR*), yellow skin (*YY*), dwarf growth habit (*dd*), but had pubescent or peach fruit (*pp*). In other words the haploid genotype as ascertained from its phenotype was accurate except for the *Pp* genes. Apparently the *p* gene in the haploid condition is not sufficient for the complete development of pubescence on the fruit, characteristic of the diploid peach type.

Having determined that these diploids breed true for simple qualitative characters, it becomes of greater interest to test them for their variability in size or quantitative characters. Since we are dealing with an absolutely homozygous genotype, the heredity of all quantitative characters is controlled, permitting an excellent determination of the effects of environment on this genotype. Accordingly the pure lines derived from the haploid both sexually and asexually were compared for relative variability in fruit size (weight) with inbred lines of certain tomato varieties and F_1 generations from them.

In two seasons, 1929 and 1930, comparative measurements were obtained from such material in which ten fruits from each plant were carefully weighed and measured. Two general comparisons were made; one between the progenies of diploids derived by self-fertilizing the haploid and those

TABLE 1. *Comparative Variability in Fruit Weight. 1929 Data*

Weight in Grams	Haploid Selfed	Diploid Regenerated from Haploid	Inbred Varieties			F_1 Generations	
			Yellow Peach	Grape Cluster	Dwarf Peach *ppdd*	Yellow Peach × New Globe	Dwarf Peach × Grape Cluster
30		1					
35	1	0	3				
40	4	23	2			1	
45	19	19	6	2		4	
50	14	5	4	7		17	1
55	2	2	2	8	1	15	2
60			1	2	4	1	6
65				1	4	1	8
70					5		3
75					2		
80					4		
Total	40	50	18	20	20	39	20
Mean (gms.)	46.5	43.3	45.8	53.2	68.7	51.8	62.5
St. Dev. (gms.)	±4.06	±4.35	±6.90	±4.80	±7.55	±4.45	±4.40
C. V. (%)	8.7	10.1	15.1	9.0	11.0	8.6	7.0

from the decapitation or callus method; and the other between the pure line diploids and inbred lines of tomato varieties bearing approximately the same fruit size.

In table 1 appear the first comparisons for the season of 1929. The

TABLE 2. *Comparative Variability in Fruit Weight. 1930 Data*

Weight in Grams	Haploid Selfed	Diploid Regenerated from Haploid	Variety Sterling Castle	Variety Gold Ball
25	1	1		1
30	19	10		3
35	39	21		13
40	33	43	2	5
45	25	18	6	
50	2	5	5	
55	1	2	11	
60			8	
65			5	
70			3	
Total	120	100	40	22
Mean (gms.)	38.0±0.34	39.5±0.38	55.5±0.85	35.0±0.53
St. Dev. (gms.)	±5.5	±5.6	±8.0	±3.7
C. V. (%)	14.5	14.2	14.4	10.6

data show clearly a slight difference in fruit weight between the two lots of diploids, one derived sexually, the other asexually. The former were significantly larger, the difference in the means of 3.2 grams being five times as large as its probable error.

The experiment was repeated on a more carefully replicated scale in 1930 (table 2). In this case the 1929 results were not verified, the two averages being practically the same. This is perhaps to be expected since there is no evident reason for any difference in the genotypes of the two lots of plants. Both should possess exactly the same chromosomes.

In tables 1 and 2 are also presented the 1929 and 1930 results, respectively, of a comparative test of the variability in fruit size (weight) of these pure line diploids and certain inbred lines of tomatoes. The latter have been inbred for periods of from three to six generations and should be reasonably homozygous. Two F_1 generations are also included.

Attention is directed to the standard deviations and the coefficients of variability. These indicate that, when only the general environment is the source of variation in fruit size, the amount of variation in homozygous lines is small. There is surprisingly little difference between the variability of the completely homozygous diploids and the inbred lines that have been made homozygous by breeding. In other words inbreeding in a naturally self-fertilized species like the tomato results in a very close approach to complete homozygosis. This is also reflected in the F_1 generations from such pure lines which are surprisingly uniform in fruit size.

The higher coefficients of variability in the 1930 data are unquestionably due to the extreme drought of that season, which not only reduced the size of the fruits but caused an uneven set of fruit.

A series of measurements of fruit shape were also taken, but inasmuch as these show exactly the same relative variability as did fruit weight, there seems to be no object in tabulating them.

Tetraploids from the Haploid

Diploid plants derived from the haploid were subjected to the decapitation process. From the callus tissue of 40 plants, 309 sprouts were rooted and grown to maturity in the summer of 1930. These were carefully observed phenotypically. Pollen from all these plants was studied under the lower powers of the microscope. Combining the results of the appearance of the plant, especially the flowers, plus the relative size of the pollen grains, it was determined that 101 or 32 percent were tetraploids. This percentage is only approximate since at least 10–15 plants could not be classified accurately, some of them apparently being sectorial chimeras of diploid and tetraploid tissue, including some other forms of abnormality.

With a little experience it is relatively easy to determine a tetraploid tomato. The leaves are noticeably thicker and broader than those of the diploid. Particularly the flowers are conspicuously large, being fully 40

percent longer than those of the standard diploid. The pollen grains of the tetraploid are larger, and more variable in size. A comparison of pollen sizes of the haploid, diploid, and tetraploid plants gives the following averages:

Haploid, 27 microns
Diploid, 27–28 microns
Tetraploid, 35–38 microns

In some of the young tetraploid plants, during the extremely hot summer of 1930, much pollen abortion was noted. When tested in the autumn some of these same tetraploid plants surprisingly showed only about 15–25 percent abortive grains. Some difficulty with the pollen determinations was encountered with certain sectorially different plants, bearing tetraploid, diploid, or abnormal areas. True tetraploids, however, uniformly possessed relatively high percentages of plump, normal-appearing pollen grains.

Despite the apparent good pollen in the true tetraploids, they set fruit with great difficulty on natural self-pollination. The seed set was surprisingly small. A cross section of tetraploid fruits, which are always smaller than the diploid from whence they came, shows almost a solid mass of woody placental or core tissue with distinct evidence of many abortive seeds. From 80–90 fruits examined, less than 20 well-formed seeds were obtained, and not all of these germinated.

These results are unlike those of Jorgensen (1928) who reports a reasonably fair seed development in his tetraploids which were derived from commercial diploid varieties. Apparently our tetraploids with absolutely identical sets of chromosomes are less fertile than ordinary tetraploids.

Cytological observations of microsporogenesis in the tetraploid tomatoes derived from the haploid show uniformly twelve sets of quadrivalent or tetrasomic chromosomes (figs. 19, 20). At diakinesis the homologous chromosomes almost invariably associate in fours, appearing in rings, strings, and figure eights. Only very rarely do any of the chromosome groups appear as disomes. In this respect our homozygous tetraploids seem to differ markedly from those of Jorgensen where the disomic condition is said to occur in the majority of the cases. In the *Datura* tetraploids, Belling and Blakeslee (1924) found that the tetrasomic condition was the rule.[2]

Chromosome counts at the metaphase of the homotypic division show an overwhelming proportion of daughter cells with 24 chromosomes. Only rarely are 23 and 25 chromosome cells evident. The entire process of chromosome distribution in both heterotypic and homotypic divisions is remarkably regular and orderly. The abnormally high sterility of our

[2] Since this was written, a paper by M. M. and J. W. Lesley (Jour. Genetics **22**: 419–425, 1930) has appeared, in which is described a tetraploid tomato showing the tetrasomic grouping in most of the chromosome sets.

tetraploids seems to be inconsistent with the great regularity in microsporogenesis. Since this point is being investigated further, there is no object in speculating on this inconsistency at the present time.

It is worth recording that the pubescent condition of the tetraploid plant and its fruit (caused by the *p* gene) is noticeably more pronounced than in the diploid. It is to be recalled that this *p* gene, when in the haploid condition, develops practically no effect so that the haploid fruit is essentially non-pubescent. Apparently this gene acts in a cumulative or additive fashion.

That 32 percent of the sprouts from the diploid callus involved a doubling of the chromosomes is a remarkably high percentage. Jorgensen (1928) reports about 5–6 percent in tomatoes with his material and his method of decapitation. Whether our higher percentage is due to the petrolatum used on the callus, to better conditions of growth, or to the fact that the diploids in our case were absolutely homozygous, is a matter of uncertainty that must be investigated later.

Summary

The haploid tomato which arose spontaneously in an F_2 generation presumably from the parthenogenetic development of an egg cell has been carried on by cuttings for five years with no evidence of mutation or change. Cytological studies prove conclusively that it possesses twelve univalent chromosomes. At microsporogenesis these univalents are distributed at random, resulting in a high degree of microspore abortion. There is no pairing of these chromosomes, proving that twelve is the basic chromosome number of the tomato.

The almost sterile haploid may be self-fertilized with great difficulty. The progeny is diploid in nature and completely fertile. Cytological observations show twelve pairs of chromosomes.

When young haploid plants were decapitated, allowing a healthy callus to form on the cut end of the stem, adventitious buds arose and sprouts developed which were rooted and grown to maturity. Approximately thirty percent of these proved to be fertile diploid plants possessing twelve pairs of chromosomes. The doubling of chromosomes must have taken place in the callus to produce plants that were completely diploid.

Histological preparations of the callus on decapitated haploid plants showed many binucleate cells. In certain of these cells a nuclear fusion seemed apparent, and in others the diploid number of chromosomes could be ascertained. While such doubling is occasionally known to occur in haploid root tissue, it does not occur in somatic, aërial tissue in the tomato unless some stimulus like wounding by decapitation is given.

The diploids thus regenerated from the haploid proved to be genetically homozygous for all qualitative characters, including fruit colors, pubescence, type of flower cluster, growth habit, and leaf type. Measurements of

quantitative characters, such as fruit shape and weight, afforded excellent determination of the relative influence of environmental agencies as they acted on a completely homozygous genotype. Data are presented on normally inbred tomato varieties and F_1 generations for a comparison of their variability with that of the absolutely homozygous diploids derived from the haploid to demonstrate the approach to homozygosity possible with inbreeding.

A large number of tetraploid plants were derived from the regenerated diploids by the decapitation method. Fully thirty-two percent of the tested sprouts from such callus tissue were tetraploid. While the pollen of these tetraploids is surprisingly good, fertility is exceedingly low. The tetraploid fruits were smaller than the diploid, and seeds were rare. Cytological studies of microsporogenesis show 48 chromosomes most of which are in a tetrasomic condition.

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EXPLANATION OF PLATES

PLATE XXXII

Microsporogenesis of Haploid Tomato. Magnification × 2133

FIG. 1. Spireme, somewhat thickened and shortened.
FIG. 2. "Stringing-out" of the chromatin material after the massing stage.
FIG. 3. Metaphase figure showing continued attachment of chromosomes.
FIGS. 4, 5, 6, 7, 8, 9. Random distribution of chromosomes in anaphase.
FIG. 10. Multipolar figure.
FIG. 11. Second division, with split chromosomes.
FIG. 12. Telophase of second division showing random distribution of chromatin bodies, and irregular formation of nuclei of unequal size.

PLATE XXXIII

FIG. 13. Diad.
FIG. 14. Hexad.
FIG. 15. Abnormal pollen grains.

Microsporogenesis in Regenerated Diploid. Magnification × 2133

FIG. 16. Strepsinema, twisting and looping.
FIG. 17. Diakinesis, conjugation of homologues.
FIG. 18. Normal metaphase plate with homologous pairs of chromosomes.

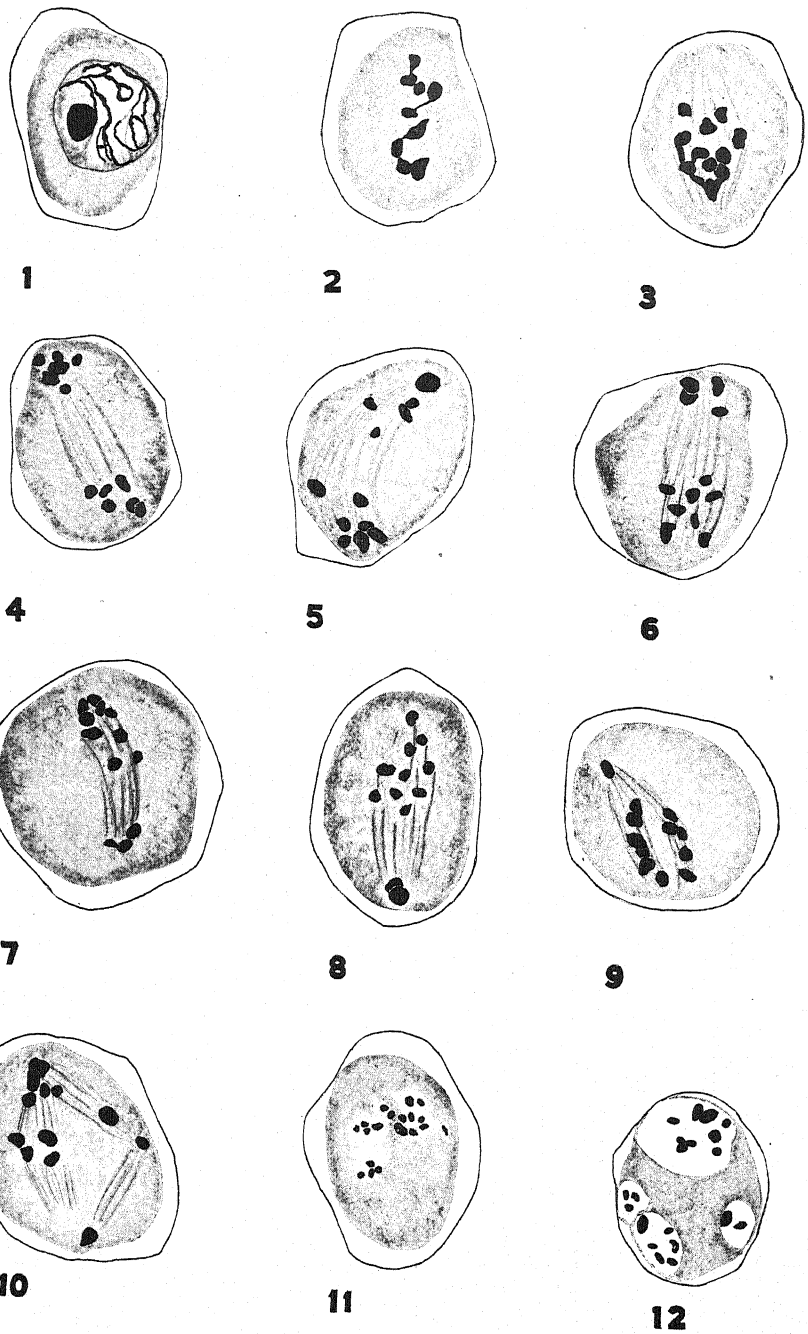
Microsporogenesis in Tetraploid. Magnification × 2133

FIG. 19. Diakinesis, showing chromosomes in groups of four.
FIG. 20. Metaphase of homotype division, showing 24 chromosomes after reduction division has been accomplished.

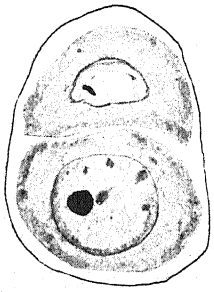
PLATE XXXIV

Haploid Callus. Magnification × 2133, Fig. 28 × 1067

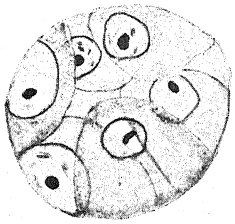
FIG. 21. Metaphase plate in haploid cell.
FIG. 22. Anaphase in haploid cell.
FIG. 23. Polar view of metaphase in diploid cell in callus.
FIGS. 24, 25. Evident failure of cell plate formation.
FIG. 26. Apparent union of two nuclei.
FIG. 27. Conjugate mitosis in single cell.
FIG. 28. Giant nucleus, probably diploid.



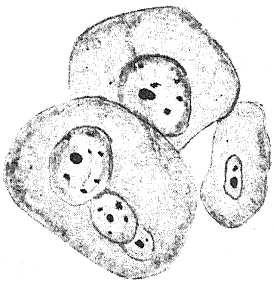
LINDSTROM AND KOOS: HAPLOID TOMATO



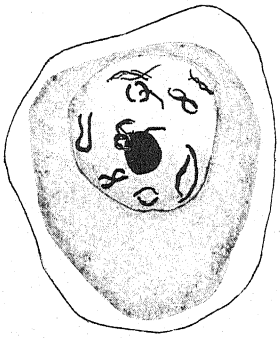
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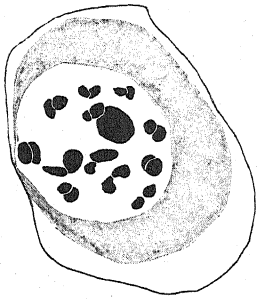
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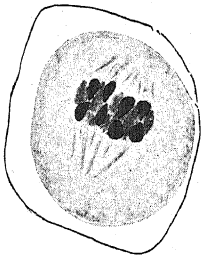
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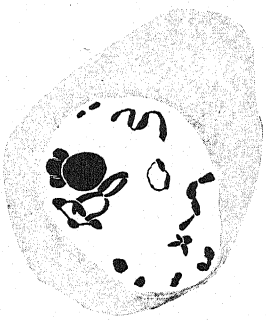
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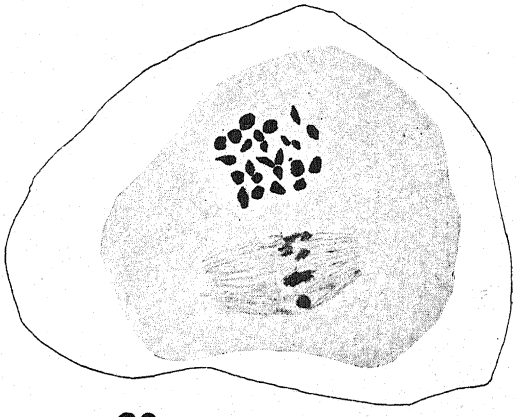
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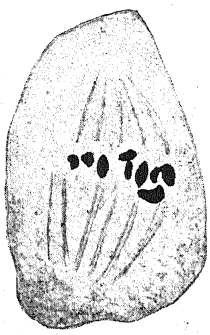


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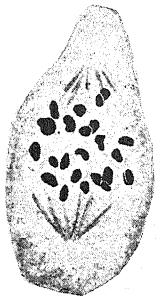


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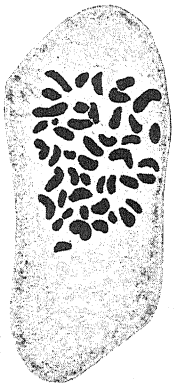
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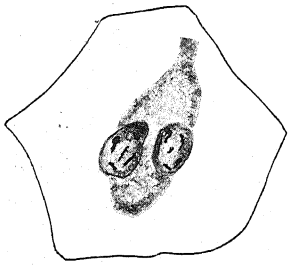
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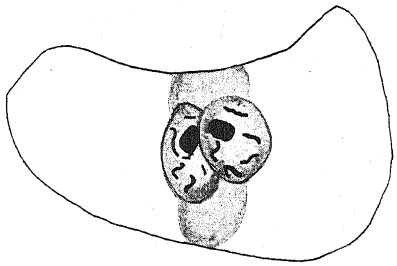
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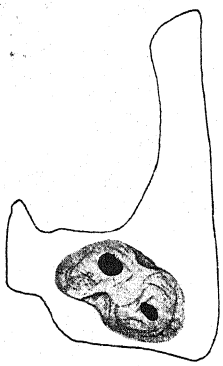
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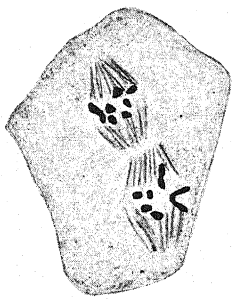
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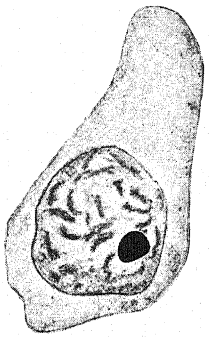
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THE GRASS GENUS *AMPHIBROMUS*

JASON R. SWALLEN

(Received for publication January 2, 1931)

While identifying a collection of Australian grasses, a specimen of *Amphibromus* was found which bore a striking resemblance to *Avena quadridentula* of South America. This led to a study of the Australian species in comparison with two anomalous South American species referred to *Avena*, *A. quadridentula* and *A. scabrivalvis*. This study showed clearly that the five species, one of Australia, one of New Zealand, one of Tasmania, hitherto undescribed, and two of South America are congeneric. The South American species are therefore transferred to *Amphibromus*.

Amphibromus has been referred to the Aveneae, but belongs rather to the Festuceae as shown by the glumes, these being much shorter than the spikelet. It is most closely related to *Schizachne* Hack.[1] but differs in the geniculate more or less twisted awns, and in the short-pediceled spikelets. The caryopsis is roughened on the surface, with a few hairs at the summit, while that of *Schizachne* is smooth and shining, and entirely glabrous at the summit.

There are few examples among the grasses of such peculiar distribution as that of *Amphibromus*, which is known only from America and Australia. There are three other genera with similar distribution, *Distichlis* and *Triodia*, found in North and South America and Australia, and *Leptoloma*, found in North America and Australia.

AMPHIBROMUS Nees

Amphibromus Nees, Lond. Jour. Bot. 2: 420. 1843; Steud. Syn. Pl. Glum. 1: 328. 1854. Nees described the genus without naming any species. However, he cites Gunn 995. This collection is the basis of *A. neesii* Steud., the first species to be described under the genus. It is therefore taken as the type species.

Spikelets several-flowered, the rachilla disarticulating above the glumes and between the florets; glumes ovate or lanceolate, acute or obtuse, often erose, the first rather narrow, 1-nerved, the second broader, 3–5-nerved; lemmas firm, lobed or dentate, 5–9-nerved, the nerves prominent, awned from the back, the awns geniculate, spreading or recurved, often twisted below the bend.

Perennial grasses, with narrow, elongate panicles, sometimes with bulbous bases. Species five, two in South America, three in Australasia.

[1] Repert. Sp. Nov. Fedde 7: 322. 1909.
Swallen, Jour. Washington Acad. Sci. 18: 20, fig. 1. 1928.

KEY TO THE SPECIES

Awn attached at or very near the middle of the back of the lemma.
  Culms erect; plants terrestrial.
    Awns 15–20 mm. long, lemmas papillose or rarely somewhat scabrous. Australasian ..... 1. *A. Neesii.*
    Awns 8–12 mm. long; lemmas densely hispid, rarely tuberculate. South American ..... 2. *A. scabrivalvis.*
  Culms decumbent at the base and rooting at the lower nodes; plants aquatic ..... 3. *A. fluitans.*
Awn attached near the summit of the lemma.
  Rachilla glabrous. South American ..... 4. *A. quadridentulus.*
  Rachilla hairy. Australasian ..... 5. *A. recurvatus.*

1. AMPHIBROMUS NEESII Steud.

Avena nervosa R. Br. Prodr. Fl. Nov. Holl. 1: 178. 1810. Not Lam. 1791. Collected by Robert Brown "apud Portum Jackson, * * *" Australia.

Amphibromus neesii Steud. Syn. Pl. Glum. 1: 328. 1854. "Gunn nr. 995. Ins. Van Diemen."

Danthonia nervosa Hook. f. Fl. Tasm. 2: 121, pl. 163A. 1858. Based on *Avena nervosa* R. Br. According to a note from the Royal Botanic Gardens, Kew, by F. Ballard, the figure is drawn from a specimen collected by Robertson (n. 546) of which spikelets have been examined. *Amphibromus hydrophila* F. Muell. is cited by Hooker as a synonym of *Danthonia nervosa*. It is a manuscript name only.

Danthonia archeri Hook. f. Fl. Tasm. 2: 122, pl. 163 B. (172 B. in text is error). 1858. "Hab. Northern parts of the Island, Gunn; Cheshunt, Archer." Gunn 995 is also cited just after the description. According to a note by F. Ballard, the plate was drawn from this collection, Hooker's sketches of the dissections being still attached to the sheet.

The characters used by Hooker to distinguish *Danthonia archeri* and *D. nervosa* are not at all apparent. The same collection, Gunn 995, is cited in both cases.

Amphibromus nervosus (R. Br.) Druce, Rep. Exch. Club Brit. Isles 1916: 604. 1917. Based on *Avena nervosa* R. Br. *Amphibromus nervosus* Nees is cited as a synonym of *Danthonia nervosa* by Hooker in his Flora of Tasmania. The name is incorrectly credited to Nees as he published only the generic name and no species.

Perennial; culms erect, glabrous, 30–125 cm. tall; sheaths smooth or scaberulous; ligule acuminate, 8–15 mm. long; blades rather firm, flat, 10–20 cm. long, 1–5 mm. wide, smaller on the innovations, scabrous above, the nerves prominent, scaberulous or nearly smooth beneath; panicles narrow, elongate, 15–40 cm. long, the lower part inclosed in the uppermost sheaths, the branches distant, ascending or appressed, the lower as much as 12 cm. long; spikelets on very short pedicels, appressed to the branches, 1–2 cm. long, 4–5-flowered; glumes acute or obtuse, more or less erose, the first 3.5–5 mm. long, the second 4–6 mm. long, 3–5-nerved; lemmas 7-nerved,

papillose, obtuse, erose, the lowermost 5–6 mm. long, awned from or just below the middle, the awns geniculate, twisted below the bend, 15–20 mm. long.

Wet marshy places throughout Australia. N. S. WALES: Temara, Dwyer in 1916. "Dubbo & Nyngan," Blakeley in 1912. Without locality, Turner in 1892. VICTORIA: Wendu Vale, Robertson 546. WESTERN AUSTRALIA: Swan River, Drummond 145.

2. AMPHIBROMUS SCABRIVALVIS (Trin.) Swallen

Avena scabrivalvis Trin. Mém. Acad. St. Pétersb. VI. Sci. Nat. 2[1]: 28. 1836. "V. sp. Chil." No specimens are cited. There is a fragment in the U. S. National Herbarium from the Trinius Herbarium labeled "Mendoza, Hooker mis. 1835."

Bromus holciformis Steud. Syn. Pl. Glum. 1: 324. 1854. "Holcus an Bromus Bertero Hrbr. nr. 329, 862. Chili." A fragment from the type from the Trinius Herbarium has been examined. The label bears the following, "Quillota Chili Hbr. Bertero n. 329, 862. Unio. Iter 1835."

Bromus? gilliesii Nees, in Steud. Syn. Pl. Glum. 1: 328. 1854. "Gillies legit in Mendoza." The type has not been examined, but the description seems plainly to indicate *Amphibromus scabrivalvis*.

Perennial, spreading by rhizomes; culms erect from a bulbous, sometimes decumbent base, 30–100 cm. or more tall; sheaths smooth, longer than the internodes; ligule 6–15 mm. long; blades rather lax, flat, 2–6 mm. wide, the lower ones elongate, as much as 40 cm. long, the upper ones reduced, some as little as 1 cm. long; panicles narrow, 6–25 cm. long, the branches ascending to drooping, often flexuous; spikelets short pediceled, 15–30 mm. long including the awns, 3–6-flowered; glumes acute or obtuse, more or less erose, the first 3.5–5.5 mm. long, 1–3-nerved, the second 4–7 mm. long, 3–5-nerved; lemmas 7–9-nerved, hispid, or sometimes tuberculate only, deeply two lobed at the summit, the lobes erose or dentate, the lower ones 5–8 mm. long, awned from or just above the middle, the awns geniculate, more or less twisted below the bend, somewhat flexuous above, 8–12 mm. long, scabrous.

Open grassland, Peru to Uruguay and Chile. PERU: Occa Pampa, Shepard 57. BOLIVIA: La Paz, Buchtien 6422. URUGUAY: Montevideo, Arechavaleta 175. CHILE: Concepcion, Neger in 1893–96; Claude Joseph 4195. Tome, Claude Joseph 4213. Temuco, Claude Joseph 4659. Cañete, Claude Joseph 4292. Santiago, Philippi 250. Mendoza, com. Hooker. Quillota, Bertero 329, 862.

3. AMPHIBROMUS FLUITANS Kirk

Amphibromus fluitans Kirk, Trans. N. Zeal. Inst. 16: 374. 1884. "Hab. North Island: in shallow waters, margins of Waihi Lake and Creek."

Perennial; culms erect from a decumbent base, 30 cm. tall, rooting at the lower nodes; sheaths scaberulous or nearly smooth; ligule 4–5 mm.

long; blades rather lax, flat, 8–14 cm. long, 2–4 mm. wide, scabrous on both surfaces; panicle 12 cm. long, partly inclosed in the upper sheath, the branches short, rather stiff, ascending; spikelets nearly sessile, 18 mm. long including the awns, 5–6-flowered; glumes obtuse, the first 3 mm. long, 1-nerved, the second 4 mm. long, 3-nerved; lemmas 7-nerved, sparsely papillose, erose, the lower ones 5–6 mm. long, awned from just above the middle, the awns straight, scabrous, 12–14 mm. long.

Floating or nearly submerged in shallow water of margins of lakes and streams. Known only from New Zealand. The only specimen in the U. S. National Herbarium was collected at Waihi Lake, the type locality, by T. Kirk. This may be a duplicate type collection.

A note from Kew says the label on Kirk's specimens bears the following information. "* * * The spikelets are developed and fertilization takes place before the panicle leaves the sheath. The spikelets fall away almost immediately."

When describing this species Kirk states that "although in very shallow water it is suberect, yet as a general rule little more than the panicle is elevated above the water. Owing to the rhachis of the spikelet being articulated below each flower, the glumes fall away almost immediately after the extrusion of the panicle from the sheath, leaving only the naked pedicels * * *."

"*Amphibromus fluitans* is usually if not invariably cleistogamous, fertilization being effected before the panicle is extruded * * * ."

4. Amphibromus quadridentulus (Doell) Swallen

Avena quadridentula Doell. in Mart. Fl. Bras. 2^3: 100. 1878. "Habitat ad Caldas prov. Minarum (Regnell III. n. 1410)."

Uralepis quadridentata Doell in Mart. Fl. Bras. 2^3: 240. 1880. "Habitat prope Rio de Janeiro (Glaziou n. 6773)."

Avena montevidensis Hack. Oesterr. Bot. Zeitschr. **52**: 188. 1902. "Montevideo, in paludosis ad Barra San Lucia, * * * leg. Arechavaleta (Nr. 283)."

Perennial; culms erect from bulbous bases, 40–135 cm. tall; sheaths scaberulous or nearly smooth, longer than the internodes; ligule 4–12 mm. long; blades firm, flat, 8–30 cm. long, 1–5 mm. wide, scabrous on both surfaces; panicles 10–35 cm. long, the branches ascending or spreading, the lower ones as much as 7 cm. long with short ones intermixed; spikelets nearly sessile, 5–10 mm. long, 2–6, commonly 5-flowered; glumes acute, the first 2–4, commonly 3–4 mm. long, 1-nerved, the second 3–5, commonly 4–5 mm. long, 3-nerved, the lateral nerves often obscure; lemmas 7-nerved, scabrous, dentate or shallowly 2-lobed, the lobes dentate, 3–5, commonly 4–5 mm. long, awned from near the summit, the awns geniculate, slightly twisted below the bend, 2–6, commonly 4–6 mm. long.

Campos and open places in woods, Brazil and Uruguay. Brazil: Minas Geraes: Serra do Caparaó, Chase 9676, 10094. Caldas, Regnell III.

1410. Rio de Janeiro: Serra de Itatiaia, Chase 8264. Without locality, Glaziou 6773. Paraná: Barigny, Dusén 15763. Rio Grande do Sul: Without locality, Dutra 401. URUGUAY: Montevideo, Arechavaleta 283.

5. **Amphibromus recurvatus**, sp. nov.

Perennis; culmi erecti 40–50 cm. alti, glabri; vaginae glabrae, internodiis longiores; ligula obtusa 5–6 mm. longa; laminae 1–14 cm. longae, 1–2 mm. latae, supra pubescentes nervis prominentibus subtus glabrae; panicula angusta 6–10 cm. longa, ramis scabris usque ad 3 cm. longis; spiculae 7–10 mm. longae, 3–5-florae; gluma prima acuta 3–4 mm. longa, 1-nervis; gluma secunda acuta vel obtusa, erosa, 4–5 mm. longa, 3-nervis; rachilla hirta; lemmata scabra, 4–5 mm. longa, 5-nervia, 4-dentata, dorso aristata, arista scabra geniculata, patente vel recurvata, 7–9 mm. longa.

Cespitose perennial; culms erect, 40–50 cm. tall, glabrous; sheaths glabrous, mostly longer than the internodes; ligule membranaceous, obtuse, 5–6 mm. long; blades flat or loosely folded, 10–14 cm. long (the upper ones reduced, 1–4 cm. long), 1–2 mm. wide, pubescent or scabrous above, the nerves prominent, smooth beneath; panicles narrow, 6–10 cm. long, the branches scabrous, appressed, as much as 3 cm. long, floriferous from the base; spikelets 7–10 mm. long, 3–5-flowered, the lateral pedicels very short, the terminal ones about 4 mm. long; first glume acute, 3–4 mm. long, 1-nerved, the second broader, acute or obtuse, erose, 4–5 mm. long, 3-nerved; rachilla hairy; lemmas scabrous, 4–5 mm. long, 5-nerved, the nerves prominent, the lateral ones extending into short teeth, awned from 1 mm. below the summit, the awns geniculate, spreading or reflexed, 7–9 mm. long, scabrous.

Type in the U. S. National Herbarium no. 1447361, collected in Broad River Valley National Park, Tasmania, in February, 1929, by R. A. Black (no. 1225).

Known only from the type collection.

This species, although closely related to the South American *A. quadridentulus* is easily distinguished by the hairy rachillas and longer awns.

It is interesting to note that each of the South American species of *Amphibromus* has a corresponding species in Australasia to which it bears a close resemblance. *A. quadridentulus* is very similar to *A. recurvatus*, and *A. scabrivalvis* to *A. neesii*.

The author wishes to express his thanks to Dr. A. W. Hill, Director, Royal Botanic Gardens, Kew, for fragments of important specimens and for notes on other specimens of which no fragments were available.

BUREAU OF PLANT INDUSTRY,
UNITED STATES DEPARTMENT OF AGRICULTURE

SOME PROPERTIES OF ALKALOIDS IN RELATION TO CLIMATE OF HABITAT

JAMES B. MCNAIR

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Since the melting points of oils and fats have been found to vary in accordance with the climate of the habitats of the plants producing them, it is of interest to determine whether a similar condition exists in the case of alkaloids.

If alkaloids have differences in melting points in accordance with their habitats, then concomitant variations would be expected in their molecular weights; percentage composition of carbon, hydrogen, nitrogen, and oxygen; structural formulae; solubility, and perhaps degree of toxicity.

CLIMATIC AND TAXONOMIC DISTRIBUTION OF ALKALOIDS

According to Boas (1927) alkaloids are found in 51 plant families. These families are classified climatically by Engler and Gilg (1919), and Willis (1925). An analysis of their data shows that 24, or 47.1%, are mostly tropical; 4, or 7.8%, are mostly tropical-subtropical; none is mostly subtropical; 5, or 9.8%, are subtropical-temperate; 6, or 11.8%, are temperate; and 12, or 23.5%, are widely distributed in habitat.

The climatic distribution of alkaloidal families may be compared with the climatic distribution of the total number of plant families. Making use again of the climatic distribution of these by Engler and Gilg, and Willis, it becomes apparent that of the 295 families of gymnosperms and angiosperms 51, or 17.2%, contained alkaloids. Of a total number of 153 tropical families 24, or 15.6%, have alkaloids; of 15 tropical-subtropical families 4, or 26.6%, have alkaloids; of 8 subtropical families 0 has alkaloids; of 7 subtropical-temperate families 5, or 71.4%, have alkaloids; of 50 temperate families 6, or 12.0% have alkaloids, and of 57 families of widely distributed habitats, 12 or 21.0% have alkaloids.

It is evident that of the plant families producing alkaloids nearly twice as many are found in the tropics as in the temperate zone. Those mostly tropical in distribution include 47.1% of all the alkaloid families, while only 11.8% are found growing largely in the temperate zone.

The ratio of tropical alkaloidal families to the total tropical families (15.6%), however, is not very much greater than the ratio of temperate alkaloidal families to the total temperate families (12.0%).

The 24 tropical alkaloidal families are: Palmae, Dioscoreaceae, Piperaceae, Moraceae, Loranthaceae, Aizoaceae, Zygophyllaceae, Simarubaceae,

Meliaceae, Anacardiaceae, Sterculiaceae, Caricaceae, Symplocaceae, Loganiaceae, Apocynaceae, Asclepiadaceae, Acanthaceae, and Rubiaceae.

The four tropical-subtropical alkaloidal families are: Gnetaceae, Amaryllidaceae, Theaceae, and Punicaceae.

No alkaloid-producing family that is largely of subtropical distribution is known.

The 5 subtropical-temperate distributed alkaloidal families are: Taxaceae, Nymphaeaceae, Papaveraceae, Buxaceae, and Campanulaceae.

The 6 alkaloidal families that are mostly temperate in distribution are: Ranunculaceae, Berberidaceae, Cruciferae, Cactaceae, Umbelliferae, and Caprifoliaceae.

The alkaloidal families of widely distributed habitats, of which there are 12, are: Gramineae, Liliaceae, Aristolochiaceae, Calycanthaceae, Leguminosae, Rutaceae, Euphorbiaceae, Aquifoliaceae, Boraginaceae, Labiatae, Solanaceae, and Compositae.

Data

The alkaloids selected for comparison in this paper do not include every alkaloidal compound but only those for which the constants have been most completely determined. The list used is that compiled by Seidell (1926). Artificial alkaloids and salts of alkaloids are not used in the calculations. The substances eliminated for these reasons are: anhydroecgonine, a derivative of ecgonine; ecgonine, a basic decomposition product of cocaine by hydrochloric acid; tropacocaine hydrochlorid, an hydrochloric acid salt of tropacocaine; arecoline hydrobromid, an hydrobromic acid salt of arecoline; emetine hydrochlorid, an hydrochloric acid salt of emetine; apomorphine, an artificial alkaloid from morphine; cotarnine hydrochlorid (stypticin), the hydrochlorid of the oxidation product of narcotine; diacetylmorphine, a salt of morphine; oxysparteine, from sparteine by slight oxidation; apoatropine, from atropine by splitting off water; and tropine, an artificial alkaloid obtained by heating atropine or hyoscyamine with baryta water.

Physical Constants in Relation to Climatic Distribution

Table 1 in conjunction with tables 2 and 3 brings out certain facts in regard to a probable relationship between climate of habitat and melting point, molecular weight, and number of carbon, hydrogen, nitrogen, and oxygen atoms in alkaloids.

Melting Points. The average melting points of the alkaloids of the various climatic groups as shown in table 1 and graphically portrayed in text figure 1 are seen to form a descending series, beginning with the tropical, to the temperate and widely distributed. Those of widely distributed habitats should form a rough average between those of the tropical and the temperate habitats, as their distribution includes both tropical and temperate climates. It is thus apparent that the melting point/climatic

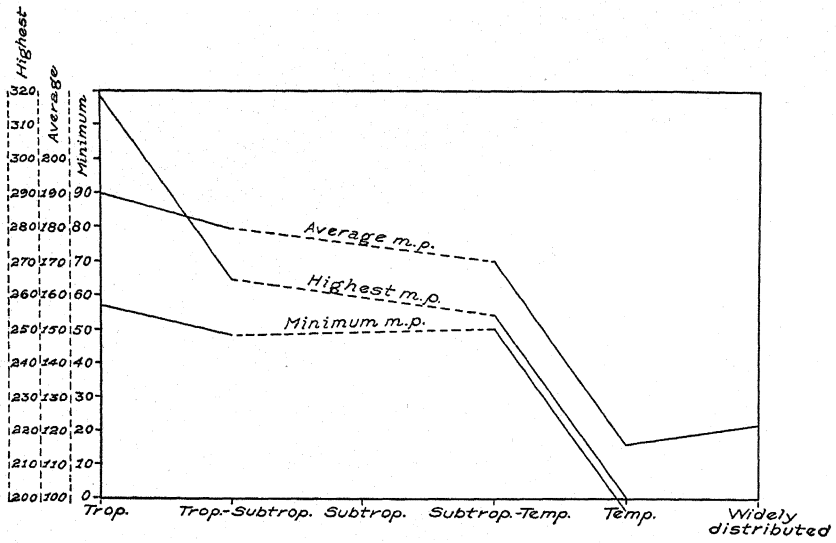
ratios are in accordance with those of the melting point/climatic ratios of the oils and fats already presented by McNair (1929, 1930).

Of the various climatic groups of alkaloids the highest melting points (table 1) form a descending curve from tropical to temperate.

TABLE 1. *Physical Constants and Maximum Doses of Alkaloids in Relation to Climate of Habitat*

Habitat	Melting Point (° C.)			Molecular Weight			Maximum Dose *		
	Max.	Min.	Aver.	Max.	Min.	Aver.	Max.	Min.	Aver.
Tropical	329	57	188	466	180	317	2.60	.002	.840
Tropical-subtropical	264	48	179	572	139	247	0.50	.036	.430
Subtropical	.....	.....	.....	.....	.....	.....	.....	.....	.....
Subtropical-temperate	254	50	170	698	230	363	0.36	.020	.258
Temperate	202	− 2.5	117	645	127	301	1.00	.001	.262
Widely distributed	250	34	121	1074	162	386	0.40	.001	.060

* In grams.



TEXT FIG. 1. Alkaloid melting points in relation to climate of habitat.

If the minimum melting points be listed for the same groups (table 1) there is formed another descending line through tropical, tropical-subtropical, subtropical-temperate, to temperate.

Table 2 groups the melting points both climatically and physically and is illustrated graphically by text figure 2. Of the tropical group the greatest number of individuals are found to have melting points ranging between 200–250° C., while the temperate group shows, as might be expected, its greatest number of individuals at a lower melting point range, namely

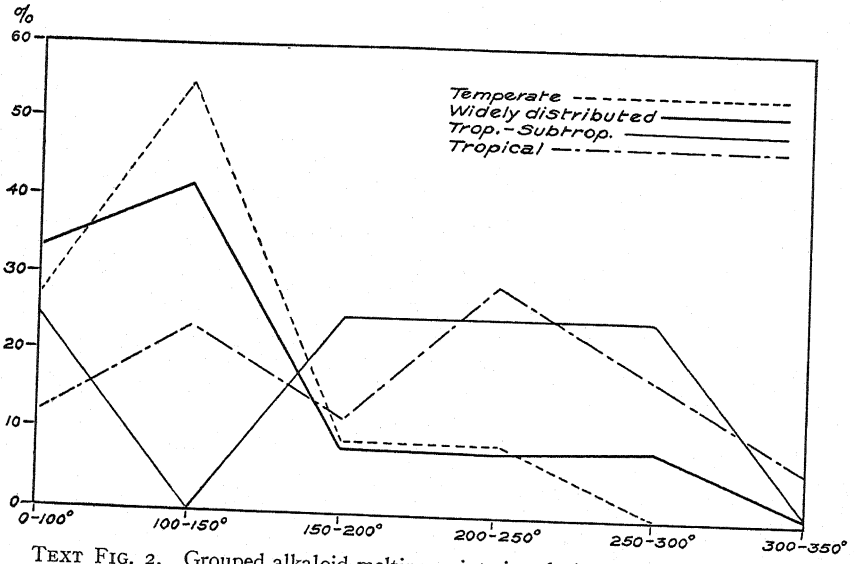
between 100–150° C. The subtropical-temperate group takes an intermediate position, that of 150–200° C., and the tropical-subtropical group

TABLE 2. *Melting Points of Alkaloids in Relation to Climate of Habitat*

Habitat	0–100° C.		100–150° C.		150–200° C.		200–250° C.		250–300° C.		300–350° C.	
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
Tropical	2	11.7	4	23.5	2	11.7	5	29.4	3	17.6	1	5.8
Tropical-subtropical	1	25	0	0	1	25	1	25	1	25	0	0
Subtropical	....	....	....	....	....	....	....	....	....	....	....	....
Subtropical-temperate	2	15.3	1	7.6	6	46.1	3	23.0	1	7.6	0	0
Temperate	3	27.2	6	54.5	1	9.1	1	9.1	0	0	0	0
Widely distributed	4	33.3	5	41.7	1	8.3	1	8.3	1	8.3	0	0

may be discarded because of the small number of representatives (four) which unduly exaggerates the relationships in this instance.

Molecular Weights. Molecular weights would ordinarily be expected to vary in accordance with variations in melting points. However, in this



TEXT FIG. 2. Grouped alkaloid melting points in relation to climate of habitat.

case, the agreement is not as close as expected. The highest, lowest and average molecular weights of the various climatic aggregates are found in table 1.

Carbon Content. The average number of carbon atoms in the alkaloids of the various climatic groups show the tropical to have a smaller number of carbon atoms in their molecules than the temperate. The maximum numbers of carbon atoms in the alkaloid molecules of the various climatic groups increases from tropical to temperate and widely distributed. With

the exception of one group the minimum carbon content likewise increases from tropical to temperate and widely distributed.

Hydrogen Content. The average, maximum and minimum numbers of atoms of hydrogen contained in the molecules of the various groups (table 3) indicate an increase from tropical to temperate.

Nitrogen Content. The average and minimum numbers of nitrogen atoms contained in the molecules of the various climatic groups show a decrease from tropical to temperate while the maximum number of nitrogen atoms contained in the molecules of the various climatic groups indicate an increase.

Oxygen Content. The average and maximum number of oxygen atoms in the molecules of the various climatic groups increases from tropical to temperate while the minimum decreases.

Molecular Structure. Alkaloids have been partially classified in accordance with various chemical groupings contained in their structures. These groupings are the pyrrolidine, pyridine, quinoline derivatives, isoquinoline derivatives, purine derivatives, imidazole derivatives, and indol derivatives.

The pyrrolidine group includes: one tropical family, Erythroxylaceae.

The pyridine group includes: 3 tropical families, Palmae, Erthroxylaceae, and Piperaceae; 1 tropical-subtropical family, Punicaceae; 1 temperate family, Umbelliferae; and two widely distributed families, Rutaceae and Leguminosae.

The quinoline group includes 2 tropical families, namely Loganiaceae and Rubiaceae; and one widely distributed, Leguminosae.

The isoquinoline derivatives include two families, one a subtropical-temperate family, the Papaveraceae, and the other a temperate family, the Berberidaceae.

The purine derivatives include one tropical-subtropical family the Theaceae, and three tropical families the Rubiaceae, Sterculiaceae, and Aquifoliaceae.

The imidazole derivatives include one tropical family, the Rutaceae, and various other families.

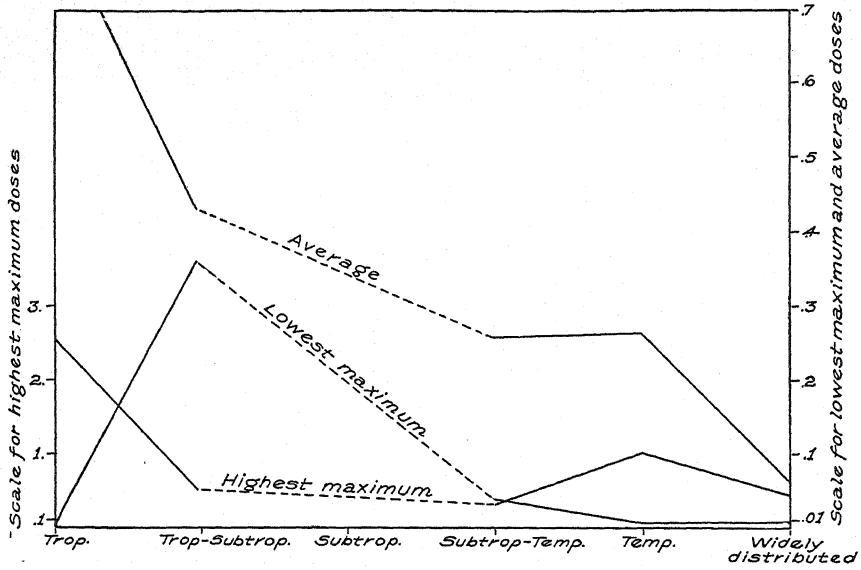
The pyridine group evidently occurs widely distributed climatically, the isoquinoline group mostly in temperate regions, and the quinoline and purine groups are found principally in the tropics.

Solubility. No consistent trend could be determined from the data at hand for the solubility of alkaloids in relation to climate of habitat. There is however a difference in solubility between the various groups. The average maximum solubilities of the pyridine group in grams per hundred grams at 25° C. are: in water, 16; in alcohol, 29; and in ether, 84. The average maximum solubilities of the isoquinoline group are: in water, 4.6; in alcohol, 8.5; and in ether, 1.9. Similarly for the quinoline group they are: in water, .09; in alcohol, 40; and in ether, 18; and for the purine group: in water, 0.95; in alcohol, 0.96; and in ether, 0.13.

Toxicity in Relation to Climatic Distribution

In table 1 illustrated by text figure 3 are found the maximum doses of alkaloids administered to man. These appear in this table associated with their respective alkaloids and climates of habitat. In this way an approximation of the toxicity of the various alkaloids can be determined according to climate.

From these figures the lowest maximum dose decreases from tropical to



TEXT FIG. 3. Alkaloid dosage in relation to climate of habitat.

temperate, the highest maximum dose decreases likewise, and the average dose similarly diminishes. It may be concluded from this, therefore, that alkaloids of tropical origin are less toxic in proportion to weight than alkaloids of temperate origin.

SUMMARY

Alkaloids are found in 51 plant families; of these 47% are mostly tropical and 12% are temperate.

The ratio of tropical alkaloidal families to the total tropical families (16%) is not far greater than the ratio of temperate alkaloidal families to the total temperate families (12%).

The curves of the average, the highest, and the lowest melting points of the various climatic groups of alkaloids diminishes from tropical to temperate. This coincides with the melting points of the oils and fats.

Of the tropical group of alkaloids the greatest number of individuals have melting points between 200° and 250° C. while in the temperate group the maximum is between 100° and 150° C.

TABLE 3. *Variation in the Composition of Alkaloids in Relation to Climate of Habitat*

Habitat	Carbon				Hydrogen				Nitrogen				Oxygen			
	Total	Average	Max.	Min.	Total	Average	Max.	Min.	Total	Average	Max.	Min.	Total	Average	Max.	Min.
Tropical	307	18	22	7	374	22	28	8	34	2	4	1	46	2	4	1
Tropical-subtropical	78	13	32	7	98	16	32	8	10	2	4	1	16	3	8	1
Subtropical																
Subtropical-temperate	312	12	42	17	350	23	54	11	16	1	2	1	63	4	8	2
Temperate	179	16	37	8	235	22	47	13	11	1	1	1	42	4	11	0
Widely distributed	312	21	54	10	478	32	92	14	28	2	5	1	65	4	18	0

The highest molecular weights of the climatic groups increase from tropical to temperate but the lowest and average molecular weights are irregular.

There is an indication that the number of carbon atoms on the alkaloid molecules increases from tropical to temperate.

The number of hydrogen atoms in the alkaloid molecules increases from tropical to temperate.

The average and minimum numbers of nitrogen atoms increase from tropical to temperate but the maximum decreases.

The maximum number of oxygen atoms increases and the minimum number decreases from tropical to temperate.

In alkaloids the pyridine group is widely distributed climatically, the isoquinoline group is confined mainly to temperate regions, and the quinoline group occurs mostly in the tropics.

No consistent trend could be determined from the data at hand for the solubility of alkaloids in relation to climate of habitat.

Alkaloids of tropical origin are generally less toxic to man than those of temperate origin.

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THE FLUCTUATION CURVE OF SEX REVERSAL IN STAMINATE HEMP PLANTS INDUCED BY PHOTOPERIODICITY[1]

JOHN H. SCHAFFNER

(Received for publication January 15, 1931)

In past experiments on sex reversal in the hemp, *Cannabis sativa* L., the writer did not succeed in producing 100 percent reversal of the individuals of any given population grown in the greenhouse during the short day period of winter (Schaffner 9, 10). The highest percentage of reversal obtained was 88+ percent for carpellate plants and 93+ percent for staminate plants. When these results were interpreted by some as indicating a multiple factor constitution of sex determination, with females, female intersexes, male intersexes, males, and supermales as the possible Mendelian combinations, further experiments became necessary to obtain definite information on the subject (see Hirata 3, 4). An experiment was devised through which "pure females" were rejuvenated after the usual blooming period and then changed to male expression in the second or rejuvenated ontogenetic cycle (Schaffner 11).

It is evidently desirable to learn how to set the ecological stage so that 100 percent of sex reversal would appear in the staminate plants in the first or usual ontogenetic cycle. A record of sex reversal in relation to photoperiodicity throughout the changing daily light period of the different seasons also seemed desirable. The aim, then, in the present experiments was to obtain, if possible, 100 percent of sex reversal of the staminate plants, including the "supermales," of a given plot; to find out how perfect the curve of reversal might be, and also to discover how definitely the percentage of reversal for any seasonal planting might be predicted.

The staminate plants only were studied because of a lack of proper greenhouse space and because the carpellate plants take a much longer period to reach maturity. The carpellate plants were mostly removed as soon as their sex reaction was definitely developed. The plants were grown on greenhouse benches with about 8 in. (20 cm.) of good, rich soil and were well watered. The temperature was kept at about 55–65 degrees Fahrenheit (13–18° C.) but the fluctuation was often considerably beyond these points. The plants were not disturbed in any way by removing flowers, branches, or leaves so whatever unusual effects were obtained were due entirely to difference of daily illumination.

Yampolsky (13) was able to cause sex reversal in staminate plants of *Mercurialis annua* by subjecting them to severe pruning, thus disturbing

[1] Papers from the Department of Botany, The Ohio State University, No. 271.

the sex balance present in the individual which under the given environment was expressing purely male characters. New methods of sex control and disturbance are continually coming to light. Recently Allan and Zotov (1) reported reversal of sex in both directions in the flowers of *Clematis foetida* caused by the presence of a rust (*Aecidium otagense*) in the inflorescence tissues and Holdaway (6) was able apparently to alter the sex ratio of the "flour beetle," *Tribolium confusum*, by a method of starvation, which was not due to differential mortality of the sexes.

The plantings were begun on July 15 and were made semi-monthly for ten months, or up to May 15. There were, therefore, 21 successive plantings all told. The general results are tabulated in table 1. Any plant that

TABLE 1. *Sex Reversal in Staminate Hemp. Greenhouse Plantings, 1929–1930*

Date of Planting	Total No. of Staminate Plants	Pure Staminate	Plants Showing Stigmas	% of Pure Staminate	% Showing Stigmas	Staminate Flowers on Reversed Plants	Flowers Showing Stigmas on Reversed Plants	% of Flowers with Stigmas	Average No. of Flowers with Stigmas on Reversed Plants
July 15.....	78	78	0	100	0	0	0	0	0
Aug. 1.....	95	90	5	94¾	5¼	365	17	4⅖	3⅖
" 15.....	69	50	19	72½	27½	898	33	3½	1¾
Sept. 1.....	114	69	45	60½	39½	1659	295	15	6½
" 15.....	38	15	23	40	60	753	140	15⅗	6
Oct. 1.....	51	12	39	23½	76½	662	165	20	4
" 15*...	10	1	9	10	90	96	69	42	7⅔
Nov. 1**...	5	0	5	0	100	20	54	73	11
" 15.....	20	0	20	0	100	75	192	72	9½
Dec. 1**...	11	1	10	9	91	55	53	49	5⅓
" 15†....	58	18	40	31	69	176	159	47½	4
Jan. 1.....	26	5	21	19	81	107	107	50	5
" 15.....	28	8	20	28½	71½	481	149	22	7½
Feb. 1.....	63	20	43	32	68	950	277	22½	6½
" 15.....	34	14	20	41	59	497	127	20⅓	6⅓
Mar. 1.....	84	48	36	57	43	1706	280	14	6
" 15.....	63	46	17	73	27	912	115	11⅕	6¾
Apr. 1.....	35	28	7	80	20	529	54	9⅕	8
" 15.....	16	14	2	87½	12½	143	15	9½	7½
May 1.....	19	19	0	100	0	0	0	0	0
" 15.....	38	38	0	100	0	0	0	0	0

* Cutworm and mice destroyed about two-thirds of the plants.

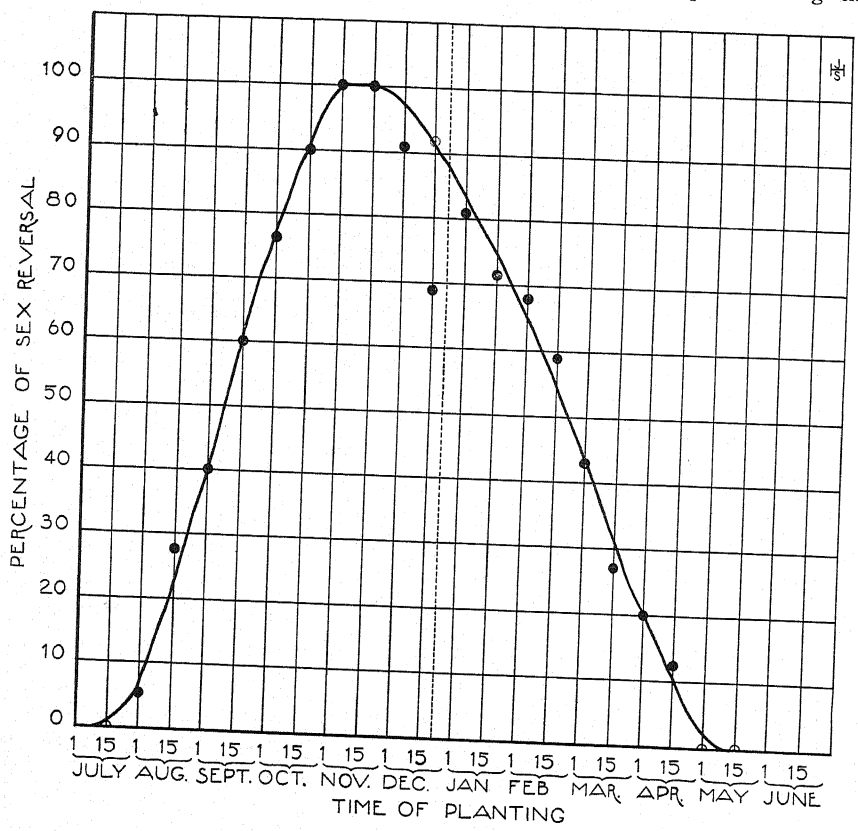
** Failed to sprout properly and nearly all died.

† Abnormal condition because of very bright weather at one period and accidental low temperature in the greenhouse.

showed flowers with fairly well-developed stigmas either on imperfect or normal ovularies or at the ends of stamens was counted as showing reversal to femaleness, since the stigma is a typical female organ. Many of the plants would never have produced normal seeds. The real problem was to find the existence of the female sex potentiality in the male plants and the ecological conditions which would swing the sex balance in the opposite

direction during the growing period after sprouting. The degree of perfection attained in the reversed sexual expression is of no special importance in these plants since it is itself dependent on the degree of action of the ecological conditions.

It will be noted that the percentage of reversed individuals rose gradually with decreasing daily illumination from zero to one hundred and then fell down again to zero with increasing daily illumination. There is one record that is decidedly below expectation, that of Dec. 15. This was plainly due to an accident which happened in the period of vegetative growth. There was an unusual amount of very bright weather at the critical period and then a very low temperature in the greenhouse room caused by the clogging up of the steam pipes for several days. A record of a previous year's planting for the same date is given as a dotted circle in the graph showing the



TEXT FIG. 1.

fluctuation curve (text fig. 1), which fits very well with the 1929–1930 curve. This record was of a planting of Dec. 15, 1920, having 42 staminate plants with 39 individuals or 93 percent showing reversal to femaleness.

Unfortunately three of the plantings show very low numbers, namely those for Oct. 15, Nov. 1, and Dec. 1. However, the expected ratio of reversal was maintained. The first reduction of the individuals was caused by an invasion of mice which injured the plants. The second and third plots failed to sprout properly for some unknown reason, so that many of the young seedlings died.

In addition to the remarkable correspondence between the daily photoperiodicity and the percentage of individuals showing sex reversal, a consistent fluctuation is also in evidence in the percentage of flowers with stigmas on the reversed plants. This rose from $4\frac{2}{5}\%$ for the Aug. 1 plants to 73% and 72% for the Nov. 1 and Nov. 15 plants and then fell down gradually to $9\frac{1}{2}\%$ for the April 15 plants. The movement of reversal shows much more irregularity in the increasing light of spring then in the autumn. This was probably due to exceedingly variable weather conditions in the winter and spring as compared with the autumn weather. A considerable difference is also noticeable between the fall and spring curves. If one could employ a proper artificial illumination without variation in intensity, an ideal fluctuation curve would no doubt be developed; or any place on the fortieth parallel where there is uniform sunlight for the proper seasons might also give ideal results.

The percentage of sex reversal under a given set of ecological conditions can be predicted with much accuracy. Any plantings between May 15 and July 15 will produce 100 percent of the staminate plants with pure male expression. Plantings from Nov. 1 to Nov. 15 will give 100 percent of the staminate plants showing a greater or less degree of femaleness. Plantings made about Sept. 5–10 or about Feb. 20–25 will give about 50 percent of the staminate plants pure and 50 percent with some degree of femaleness expressed.

As reported in previous papers on sex reversal, all sorts of abnormalities in floral structure are developed, which is to be expected because the tissues from which the flowers and floral parts are produced may be made up of mosaics of cells in various sexual states in various degrees of development. Among the abnormalities observed were the following:

1. Flowers with helicoid stigmas.
2. Structures with the texture of stigmas but representing neither ovulary nor stamen.
3. Pollen-bearing stamens ending in a single stigma at the tip.
4. Imperfect ovulary on one side with two stigmas and two nearly normal microsporangia on the other side.
5. One vestigial ovulary with a perfect stigma.
6. An imperfect ovulary with two stigmas.
7. A nearly perfect ovulary with three stigmas, one smaller than the other two.

8. A flower with one imperfect ovulary with two stigmas, one imperfect ovulary with one stigma and a normal microsporangium at the side, and with one normal stamen which, however, had a stigma at the tip.

9. A flower with a fairly well-developed ovulary with two stigmas considerably below the apex and with the apex occupied by a large ovoid stigmatic mass.

10. Ovularies with three stigmas.

11. A flower having a stamen with an imperfect stigma appendage at the base on the inner side.

12. A stamen with an imperfect ovulary and single stigma on the inner side near the base.

One odd reversed staminate plant was studied in detail. It had 15 pure staminate flowers, 16 flowers with stigmas, and 5 flowers with normal ovularies and stigmas without a trace of stamen vestiges. It first had normal staminate flowers below; then it began to reverse, having staminate flowers with stigmas; and at the very last, at the tip of the inflorescence, several normal carpellate flowers developed with the normal carpellate floral sheath. This is the reverse of what usually occurs. In the carpellate plants reversal from femaleness to maleness usually comes late in the ontogeny, while in the staminate plants normally the reversal to femaleness comes early and in the later stage of the ontogeny the tissues produce only staminate flowers. This individual was, therefore, just the opposite in reversal behavior from the usual condition. The reversal was from normal male expression to normal female expression as the ontogenetic gradient developed. In past experiments with carpellate plants, cases have developed in which the individual progressed to complete staminate expression. The female state was changed completely to the male state in the advanced stage of maturity (Schaffner 8).

According to recent cytological evidence presented by Hirata (5) hemp has the common type of allosome distribution, namely a homomorphic set (AA) for the carpellate plant and a heteromorphic set (AB) for the staminate plant. But such difference in chromosome constitution does not imply any direct relation to sex determination. To say that it does, according to our present evidence from both plants and animals, is a mere assumption based on a very imperfect correspondence. Even if the correspondence were complete we would still have to have some sort of positive proof before making such an hypothesis. Such proof has not been produced, but practically all of our experimental and taxonomic evidence indicates exactly the opposite. After sex reversal has taken place there is no correspondence, for then the female tissues show an AB set of allosomes and the male tissues an AA set of allosomes. The sexual state therefore is dependent on the determination of physiological balance independently of the allosomes.

Whenever there is a sufficient change in the ecological condition, the physiological reactions change and with them the sexual state and expression. The allosomes seem to have nothing to do with the matter. They are entirely passive. Since this is the experimental evidence and is in harmony with the passivity of the allosomes in relation to secondary and primary sex determination of the gametophyte we are fully justified in assuming that they are also passive in the egg when the sex of a dioecious sporophyte is first determined.

The production of 50 percent pure male plants and 50 percent male plants with a partial reversal to femaleness at the planting periods of September 5–10 and Feb. 20–25 is just as definite as the production of approximately equal numbers of males and females in the decidedly uniform environment and determinate physiological gradients in the seeds. In the first case there was no shifting of allosomes or hereditary potentiality associated with the reversed determination; in the second case there was a definite association corresponding with the sexual state ascertained at a later period. The problem is to find out in what way and by what means this correspondence is brought about. Since the sex determination in the 50 percent of reversed individuals was accomplished in spite of the fact that supposed allosome determiners for a contrary result were present and were not changed nor shifted, there is no evidence that the allosomes acted as determiners previously. The whole evolutionary sequence of the time of sex determination would bring us to the same conclusion. Our knowledge of plant evolution indicates that allosomes are but one result of the evolution of unisexuality and have nothing to do primarily with the determination of differential sexual states and structures, because these were completely established long before unisexuality appeared. Furthermore, if the allosomes or any supposed sex-determining units which they might contain really determined sex, then sex reversal would be impossible and the carpellate hemp, which possesses a homomorphic set of allosomes and is thus claimed to be homozygous for sex, could not show maleness unless this were introduced into it from some outside source. When gametes are developed from haploid gametophytes or directly through reduction from diploid male and female normal or reversed individuals, their primary sexual states and structures do not follow their allosome content but are determined by the previous sexual states of the tissues from which the gametes are derived. There is no correspondence between specific allosome condition and the primary sexual states and peculiar sexual morphological differentiations characteristic of the egg and sperm; yet these are certainly due to differential sexual states.

Summary

Taking advantage of the difference in length of daylight from July 15 to May 15, a series of bimonthly plantings, in the greenhouse, of *Cannabis sativa* was carried on in order to find out the fluctuation in sex reversal of

staminate plants as induced by photoperiodicity. A very perfect fluctuation curve was developed, ranging from zero reversal for the July 15, May 1, and May 15 plantings to 100 percent reversal for the Nov. 1 and Nov. 15 plantings. There are no super-males in respect to reversal, since every staminate plant can be caused to express femaleness. The degrees of readiness or difficulty of reversal shown by different individuals are to be ascribed to degrees of differentiation, both physiological and morphological, which the individuals have attained.

The determination of sex in the hemp is not caused by an allosome differential, but these chromosomes must be assumed to play a passive rôle. Determination is brought about through differential physiological states developed in gradients in both the egg and the vegetative body.

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ROCKY MOUNTAIN HERBARIUM STUDIES I.

AVEN NELSON

(Received for publication January 18, 1931)

The following studies cover a few of the interesting plants that have been accumulating over a period of some years. For lack of time, publication has been practically at a standstill but the field work in the Southwest, primarily in New Mexico and Arizona, has become with the writer an avocation to be indulged in whenever a vacation from the classroom is available. Besides the writer's collections, other material from various correspondents is at hand, thus making comparisons possible. Proposed novelties, therefore, do not ordinarily rest upon single collections. Many of the descriptions were drawn up long since but have been rechecked and modified where additional material made fuller diagnoses possible. It is the writer's purpose to follow this paper with others of a like nature to be based upon much other material not yet covered. As soon as possible, duplicate material will be made into sets for distribution to the leading herbaria.

NEW SPECIES FROM MOUNTAINS AND DESERTS

Talinum eximium, n. sp.

Root large, deep-set, semi-woody, fusiform tuber-like and presumably branched below, surmounted by one or more short caudex-like stems one or more cm. long, which bear a few short very leafy branches; leaves fleshy-cylindric, broadly linear when dry, 12–20 mm. long; floral branches scape-like and bracteate, only 1–3 cm. long, arising between or at base of the leafy branches and not much longer, these bearing a bracteate few-flowered cyme: flowers large and showy, pink or lavender-purple; sepals ovate but with abruptly acute apex, about 7 mm. long, and lengthening in fruit; pale-green, with narrow scarious margin; petals narrowly obovate-oblong, margin nearly entire, about twice as long as the sepals; stamens 30 or more, in groups, the base of the filaments more or less coherent; styles early deciduous, about as long as the ovary, completely united, the stigmas appearing subcapitate; capsule oval, the three oblong valves dehiscing throughout; ovules many in an oval cluster that appears stipitate after the floral parts and the valves have fallen.

Collected by Mrs. Gladys Convis, stationed at the Carlsbad Caverns, New Mexico. This is her no. 56, secured May 1930. This genus has furnished several novelties in recent years, those most nearly allied to this one being Dr. Greene's *T. validulum*, Leaflets 2: 170. 1913, and *T. pulchellum* Woot. & Standl., U. S. Nat. Herb. 16: 121. 1913. (Type R. Mt.)

Potentilla brevifolia Nutt. var. **perseverans**, n. var.

A slide rock plant on the high peaks, with root of indefinite length crowned by a caudex of few to several subterranean woody branches (stems) that lengthen year by year keeping the terminal bud or buds at the surface of the deepening wash; these caudex branches are enlarged rough and brown by the persistence for several years of dead leaves and flower stalks as they become gradually buried: leaves small, 10–12 mm. long, murky-green and scurfy-glandular, crowded on the crowns, the petioles from very short to 2 or 3 cm. long; blade mostly 5-pinnate; the pinnae closely approximate or overlapping, subreniform being broader than long, often decidedly inequilateral, the base truncate-subcordate, deeply bi-cleft and then irregularly 3–5 toothed or lobed, the lobes rounded-obtuse: the herbaceous flowering stems from very short to 1 dm. in length, naked, or foliar bracted, bearing a small crowded cyme of medium size golden-yellow flowers: sepals oblong, subacute, longer than the obtuse bractlets; petals broadly obcordate, exceeding the sepals.

Though this variety is strikingly different in aspect from *P. brevifolia*, its floral characters link it to that species.

It was secured by Frank M. Hurdle, number 34, on Twin Peaks, in the Challis National Forest (Idaho), August 1928; also by Nelson and Macbride, no. 1976, Jarbidge, Nevada, July 8, 1912. (Type R. Mt.)

Amsonia arizonica, n. sp.

Glabrous perennial from a low woody branched caudex, the annual stems numerous and crowded, simple erect leafy 3–4 dm. high: leaves narrowly oblong, tapering to the acute ends, almost sessile, very short at base, gradually longer upward, some as much as 5 cm. long and 6–8 mm. broad: thyrsus sub-capitate, the flowers few-several (7–12?), the calyx-lobes, 6–8 mm. long, lance-linear, long-attenuate, the attenuations pectinate with flagellum-like teeth or hairs; corolla greenish-blue and greenish-purple with pale lobes, 15–18 or even 20 mm. long, the lobes ovate, 2–3 mm. long, the tube proper narrow, less than half of the total length, enlarging toward the middle and reaching its maximum expansion at the insertion of the anthers just above the middle, tapering slightly toward the orifice but scarcely constricted, densely ciliate-hairy at the orifice (the hair not reflexed) and downward through the relatively long throat to the anther ring; below the ring a coarser matted reflexed pubescence: the large stigma in contact with the base of the anthers, the collar inconspicuous, the body low and the lobes so short and fused as to be demonstrated with difficulty: fruits not known.

The only known species to which this may be closely related is *A. Palmeri* Gray. Should its mature follicles prove to be torose, that would ally it to *A. brevifolia* Gray. In either case it is distinct by reason of its unbranched stems and its floral characters. The pectinate calyx lobes, the long throat with open orifice, the low insertion of the stamens and the nearly simple stem.

Type material from low stony sand-bar creek banks, about twenty miles south of Ashfork, Arizona, on the Ashfork-Prescott road, April 28, 1925, Aven Nelson, no. 10247. (Type R. Mt.)

Also from Mule Mts., Arizona by L. N. Goodding, April, 1909, no. 57.

Amsonia filiformis, n. sp.

Canescently lanate throughout (including calyx-lobes), strongly so on the lower part of the stem: stems evidently clustered on the crowns of a low caudex, only 1–2 dm. high, the base of each simple, corymbosely branched upward: leaves numerous, linear-filiform, 1–3 cm. long, the sterile shoots and their leaves overtopping the compact low corymb: calyx-lobes linear, about 3 mm. long; corolla-tube proper slender, expanding into an urn-shaped throat of equal length, the whole tube 10 mm. long and the ovate-lanceolate lobes 5 mm. long; stamens seated in a dense ring of fine spreading hairs which close the constricted orifice of the urn, the walls of the expanded throat covered with a dense minute reflexed pubescence which is gradually eliminated in the tube; stigma showing three distinct regions,—the reflexed collar, the low broad, middle disk, and the two very short stigmatic lobes.

This proposed species is based upon specimens collected in the state of Chihuahua, Mex. by C. G. Pringle, April 9, 1898, no. 6796. (Type, R. Mt.) The label bears the additional notation: Gravelly plains near Lake Guzman, 4000 ft. It was distributed as "narrow-leaved form" of *A. tomentosa* Torr.

Phlox Woodhousei oculata, n. subsp.

Perennial from a woody root with an enlarged crown (sometimes short branched) giving rise to one or more (few) stems which generally are less than 1 dm. high: leaves crowded below, on internodes so short that the leaves appear almost rosulate or whorled on the sterile shoots, glabrous, slightly thickened and sub-glaucous, with narrow cartilaginous margins, oblong-linear, sessile or with clasping sub-connate base, abruptly apiculate, 15–30 mm. long, 2–4 mm. broad, upwardly becoming sparsely glandular-pubescent; stems glandular-pubescent, increasingly so upward and in the inflorescence: flowers a beautiful pale-purple or, apparently sometimes blue, in short cymes, sometimes simple but more often crowded-paniculate: pedicels slender, variable but at length longer than the calyx: calyx very glandular-pubescent, about 10 mm. long, the lance-linear teeth about half as long as its tube; the corolla tube surpassing the calyx by one half, the widely spreading lobes about 10 mm. long, cuneate-obovate, deeply cordate-emarginate and with a strikingly beautiful occulus formed from the yellow throat and a pair of irregularly radiate very dark purple spots on the base of each corolla lobe (these dark spots show on the exterior also); stamens equally inserted, all the anther tips showing just above the calyx tips; the pistil only half as long as the calyx, the slender stigmas longer than the style and the latter about as long as the ovary.

This interesting plant may be worthy of specific rank but since our knowledge of *P. Woodhousei* (Gray) E. Nels. is far from satisfactory, it seems best for the present, at least, to hold it as a variation only,—an interesting and easily recognized one by reason of its oculate corolla and the nearly glabrous leaves. Dr. Brand (in his Monog. Polem. 74. 1907) speaks of the leaves of *P. Woodhousei* as densely pilose. He follows Gray in retaining *Woodhousei* as a var. of *P. speciosa*, with which it has little in common except the short pistil.

Excellent material of the new variety was secured at Cooley in the yellow-

pine belt of Arizona. It was abundant in moist places in the open woods, near Cooley Lake; Aven Nelson, May 6, 1925, no. 10357. (Type, R. Mt.)

Phlox cortezana, n. sp.

Subligneous at base but the distinctly woody branched caudex scarcely emergent from the soil, the current season's stems few–several, loosely tufted, 10–15 cm. high; pale or whitish, leafy, the bark slightly shreddy on the older stems and softly crisped-pubescent on the younger: leaves subglabrous to softly crisped-pubescent, green, broadly linear, more or less curved-spreading, 2–4 cm. long, 2–4 mm. broad, the margin with a nerve-like border resembling the mid-rib in size and color, short-cuspidate at tip: inflorescence a paniculate-cyme of few–several flowers on each stem, the crisped pubescence more noticeable than on the stem and leaves: sepals linear-cuspidate, with stout mid-nerve, green except for the scarious-plicate margins below the middle, 7–9 mm. long: corolla varying from white to blue or pink, salverform, when fully developed its tube nearly twice as long as the sepals, its obovate lobes 5–7 mm. long, obscurely crenulate and often slightly retuse.

The center of distribution for this species is somewhere in southwestern Colorado. It has been collected a number of times and will be found in herbaria under one or another of the following names: *P. longifolia*, *P. Stansburyi* or *P. cernua*, to all of which it is allied. It is, however, satisfactorily distinct as evidenced by the key which separates it by characters and by its geographic distribution. The following, all from Colorado and adjacent Utah, are representative. COLORADO: On sage brush plains and slopes, bordering the highway from Cortez, Colo., to Mesa Verde Park, Aven Nelson, May 11, 1925, no. 10436 (Type, R. Mt.); Cortez, May 10, 1925, Aven Nelson, no. 10383; Palisades, Mesa Co., May 14, 1898, C. S. Crandall; Cimarron, May 18, 1898, C. S. Crandall, no. 1586; Pagosa Springs, 1924, Hazel M. Schmoll, no. 1093; Cerro Summit, June 7, 1901, C. F. Baker, no. 60; Montezuma Forest Reserve, 1906, H. N. Wheeler, no. 2630; Glenwood Springs, June 17, 1899, Geo. E. Osterhout. UTAH: Millford, June 5, 1902, L. N. Goodding, no. 1033; Fort Duchesne, 1913, Mary R. Carter, no. 18.

Phlox visenda, n. sp.

Shrubby at base with gray furrowed bark, the branched woody caudex sometimes 1 dm. or more high, the season's stems suberect, crowded, usually 2 or more from each branch of the caudex, these rarely 1 dm. long, very leafy and terminating in a leafy crowded (almost subcapitate) cyme; stems at base whitish or stramineous, becoming green and glandular-pubescent upward: calyx-lobes about 1 cm. long, pubescent with variable more or less flattened jointed hairs a large part of which are minutely gland tipped, linear-lanceolate, green, the overlapping scarious margins of the tubular portion not noticeably carinate; corolla mostly pink, fading to a blue, its tube $\frac{1}{2}$ longer than the calyx, its lobes narrowly obovate, somewhat emarginate, 6–8 mm. long; stamens unequally inserted, the anthers of the higher ones slightly protuberant; the tips of the stigma reaching the corolla orifice.

The species is based on the writer's no. 10219 (Type, R. Mt.) from the Grand Canon, Ariz., Collected on April 27, 1925. It occurred in swales in open woods and seemed to be rather abundant. Apparently it is the same as *P. longifolia brevifolia* Gray; *P. Stansbury brevifolia* Brand. In raising it to a species a new name seems advisable because of the earlier *P. brevifolia* Baumann.

Collomia Hurdlei, n. sp.

A subalpine slide-rock plant with slender taproot of variable length depending upon the age of the plant and the extent of the "wash" that has gradually covered it: stems many, from the small crown, slender (almost filiform), the length determined by the depth of the crevice or the breadth of the shale slab from under which they protrude: leaves crowded on the aërial portion, the uppermost somewhat involucrating the terminal flower cluster, entire, petiole shorter than the blade which varies from oblanceolate to spatulate-oblong, those on the sterile shoots rosulate and nearly oval and sessile, none more than 2 cm. long and most of them much shorter: flowers few–several, crowded, white or streaked with pink or purple: glandular-pubescent in the inflorescence, obscurely so if at all on the sordid foliage; calyx about 8 mm. long, the turbinate tube nearly as long as the ciliate-glandular broadly linear lobes; corolla 15–20 mm. long, the slender tube gradually expanded into the funnelform limb which is cleft below the middle; the obovate-truncate-lobes barely crenately toothed; stamens shorter than the corolla-lobes; the stigma sometimes showing.

As pointed out by the late Dr. Edwin B. Payson in his paper on "*Collomia debelis* and its relatives" (Univ. Wyo. Publ. Bot. 1: 79. 1924), this group of species divide evidently into those with *exserted* stamens and those with *included* stamens. In setting up two forms with included stamens he made them varieties of *C. debelis* (typica) a species notable for its exserted stamens, but expressed the opinion that these two when better known, may seem worthy of specific rank."

Now that a definitely different form has come to light with included stamens it seems desirable to follow up his suggestion that these be separated from the *C. debelis* group. I am therefore naming this new species for its discoverer and transferring the two varieties to it since they are clearly of this alliance rather than of *C. debelis*.

The specimens of the new species were collected on Twin Peaks, in the Challis (Idaho) National Forest, by Mr. Frank M. Hurdle while serving as "Forest Lookout." For the sake of ready reference we will call it number 35, August 1928.

Collomia Hurdlei A. Nels., var. **integra** Payson, new comb.; *C. debelis*, var. *integra* Payson, l.c. 83.

Collomia Hurdlei A. Nels., var. **trifida** Payson, new comb.; *C. debelis*, var. *integra* Payson, l.c. 85.

Both of the above varieties are distinguished from the species by the grayish puberulence of the herbage and the tendency of both to toothed or trifid leaves.

Salvia summa, n. sp.

Several–many stemmed from a perennial root, the stems simple or sparingly branched, assurgent, 1–3 dm. long, finely and densely silky-ciliate especially below, upwardly the pubescence becomes short and semi-hispid and the surface of stems and leaves sprinkled with resinous atoms, that of the leaves and especially the petioles showing similar gradation from below upwards: basal and lower stem-leaves often pinnately lobed (occasionally to the midrib), the terminal lobe coarsely toothed, relatively large; the other stem leaves ovate to cordate-triangular and variously toothed: flowers showy, blue, mostly singly in the axils of the leaves (two at each node), short-pedicelled, calyx 2-lipped, to the middle, the upper lip three lobed, the middle one triangular and shorter and broader than the lance-linear outer ones; the lower lip of two lance-linear lobes similar and equalling the outer of the upper lip, pubescence of calyx and corolla similar to that of the subtending foliar bracts and stems; corolla 25–35 mm. long, more than 3 times as long as the calyx, widely 2-lipped, the tube slender but gradually dilated to the spreading lobes, the upper lip concave and nearly or quite entire or abruptly acute, the lower usually bifid; connective shorter than the filament, its branches unequal, both anther cells polliniferous; the unequal subulate stigma lobes well exserted and surpassing the stamens; nutlets smooth.

This species belongs in Bentham's section of the genus *Heterosphace* and is related to both *S. Roemeriana* Scheele and *S. Henryi* Gray. It differs from both in the color of the flowers, from the former in being smaller, less coarse, and with smaller less angulate-toothed leaves and in having the pubescence of the latter, though lacking its ciliate-bearded calyx throat and sinuses, and in its conspicuously pinnate angulate lobed leaves.

No. 59, by Mrs. Gladys Convis in May 1930, in rock crevices near springs. Carlsbad Caverns, New Mexico. (Type, R. Mt.)

Verbena verna, n. sp.

Pubescent throughout, with irregularly spreading or even reflexed long ciliate hairs, more densely so upward, especially in the inflorescence: biennial or short-lived perennial, the stems usually two or more from the crown of a semi-woody taproot, simple or branching above, erect or spreading (if long, decumbent at base), 2–4 dm. long, main axis and branches each bearing a dense subcapitate spike: leaves with short broad somewhat margined petioles (the uppermost nearly sessile), mostly ovate with abruptly contracted base, from merely toothed to irregularly incised and toothed, the largest up to 5 cm. long, and two-thirds as wide: peduncles medium stout, 3–6 cm. long; calyx densely long-ciliate-strigose, practically sessile, longer than the lanceolate bract, keeled on the midribs of the acuminate lobes, about 8 mm. long; corolla purplish or shading to blue, not twice as long as the calyx, stigma just protruding from the hairy throat; nutlets cylindric, 2–3 mm. long, pitted-reticulate.

Specimens examined: Diamond Creek Canyon, northern Arizona, Norman C. Wilson, no. 95 (Type, R. Mt.), April 1893; Yucca, Ariz., Marcus E. Jones, no. 3901, May 14, 1884; La Cunega, Sonora, Mex. Leslie N.

Goodding, No. 952, July 18, 1911. Another collection, strikingly similar except for the pinnately cleft leaves is no. 4254 of Munz, Johnston and Harwood, Providence Mts., Mojave Desert, May 21–24, 1920. This may be designated as **V. verna fissa,** new variety.

This may be considered as a segregate from *Verbena ciliata* Benth. Pl. Hartw. 21, 1834. Contrasting the two we have in *P. verna* a taller erect plant as against a "low depressed" one; a soft ciliate pubescence as against "hirsute or hispid"; leaf-blades toothed or cleft as against "3-parted and again incised"; linear-acuminate calyx-lobes as against "short subulate" teeth; pitted-reticulate nutlets with the linear ventral scar brown and smooth as against "reticulate rugulose" and the ventral white scar also rugulose.

The Hartweg type came from the plains of Western Texas and while later authors have applied the name to an increasingly diverse group, it seems doubtful if we should continue to modify the descriptions in order to cover the allies made known through later collections.

Ruellia Gooddingiana, n. sp.

Green in aspect, glabrous except for the scattering white flattened hairs on the stems, petioles and leaves, especially on the veins (a lens shows on the lower leaves a dense subscabrous obscure pubescence), in the inflorescence it becomes finer, denser and glandular-viscid: stems one or more from a woody subterranean caudex, 2–5 dm. high (including the paniculate inflorescence); leaves ovate-oblong, obtuse, the radical much smaller than the stem leaves, the larger of these 10–15 cm. long: calyx 5-parted, the lobes narrowly linear, erect, becoming 2 cm. or more in length; corolla faded (possibly whitish or pale blue), its slender tube a half longer than the calyx, abruptly widening into the funnel-form limb, which is 10–12 mm. broad, stamens almost equal with oblong-linear anthers; the stigma lobe flattened, oblong, the posterior lobe a tooth-like vestige.

The specimens at hand were distributed by L. N. Goodding, no. 959, from La Cienga, Sonora, Mex. (probably near the international border, not far from Bisbee, Arizona). They bore the additional notation "Under Mesquite."

It is evidently closely related to *R. tuberosa* L. but that, of course, if it comes into the U. S. at all, will be found only on the Gulf coastal plain, while the species now described is of the dry interior, the Arizona-Sonora region. That it may be *R. tuberosa longiflora* Gray is possible although there is nothing in *R. Gooddingiana* to suggest a "velvety pubescence."

Pentstemon connatifolius, n. sp.

Herbage glabrous and somewhat glaucous, obscurely scurfy, pale-green with a tendency to splotching with purple, 4–8 dm. high: stems simple below, more or less branched above, leafy throughout: mid-stem leaves largest, these usually completely connate-perfoliate and then the two together forming a narrowly oval to narrowly elliptic leaf-body 10–15 cm.

long, often, however, each leaf ovate, tending toward acute and then barely connate or even abruptly short-petioled; upward from the middle the leaves are gradually smaller but perfoliateness usually persists even in the foliar bracts; downward from the middle the leaves become smaller and gradually distinct, the basal on stoutish margined petioles as long as the blade; all of the leaves sharply serrate-dentate, the teeth varying from minute to medium large and then doubly serrate: the cymose-panicle comparatively small and few flowered, practically glabrous, usually purplish or even a rich purple on pedicels and calyx margins: calyx short (less than 5 mm.), the sepals ovate, acute or sometimes merely abruptly cuspidate: corolla red, changing toward purplish-red in drying, obscurely glandular-puberulent inside and out, tubular, 20–25 mm. long, expanding gradually into the narrow throat and the short limb of five suborbicular lobes; anthers and sterile filament glabrous.

This at first glance suggests *P. spectabilis* Thurb., and, indeed, it belongs in that Section. To contrast the two: *P. spectabilis* is floriferous for half its height, the stout peduncular cymes arising from leaf-axils and continuing indefinitely in diminishing foliar bracts; the corollas are large, expanded abruptly into a broad throat 3–4 times as long as the short tube; the flowers are pale-lilac to purple: in *P. connatifolius* the inflorescence is a short compound cyme terminating the branches; the smaller corollas are tubular throughout, the tube proper being half of the length; the flowers are definitely red, in the living state.

On sandy dry slopes, "Apache Trail," Salt River Canyon, not many miles from the Roosevelt Dam, Arizona. Secured by the writer, May 3, 1925, no. 10314. (Type, R. Mt.)

Pentstemon exsertus, n. sp.

The simple stems few, from a brittle subterranean caudex, puberulent (under a lens) as are also the under-surfaces of the leaves, 3–4 dm. high, leafy to the middle, the upper half of the plant constituting the narrow naked paniculate cyme: leaves variable in size but all of them oblong-lanceolate, in length up to 1 dm., on petioles shorter than the blade, those of the current year's crown mostly very small: bracts small, lance-acuminate: calyx dark-green, purplish or red; the sepals broadly lanceolate, thick with subcartilaginous margins, often finely nerved, 5–8 mm. long: corolla scarlet, glabrous, about 25 mm. long, tubular-trumpet-shaped, with oval-oblong lobes; sterile filament glabrous as are the conspicuously exserted anthers, the slender filaments often surpassing the corolla by half its length: anthers horse-shoe shaped, the anther cells obversely saccate; *i.e.* open to the free tips and saccate at the connective end.

This seems to be unusual among the scarlet Pentstemons, by reason of the exserted anthers, and the obversely saccate anther cells. The aspect of the plant is characteristic also, in its leafiness from mid-stem down, and its naked slender floral portion from mid-stem up.

Secured in Salt River Canyon, on the Apache Trail, about 25 miles below the Roosevelt Dam. Aven Nelson, no. 10624. (Type, R. Mt.), May 4, 1925.

Castilleja galeata, n. sp.

Perennial, probably short-lived; the weedy-looking bases of the surviving stems dying back and breaking some 5–10 cm. from the surface of the ground, the next year's stems springing from these stumps (often two or more from near the top); the current year's stems 3–4 dm. high, slender, stramineous, more or less cinereous with stiffish spreading hairs having reflexed tips: leaves less pubescent, numerous, small, dark-green (contrasting sharply with the straw-colored stems), 15–20 mm. long, narrowly lance-linear with 2–4 or more variable linear pinnae (often mere teeth); floral bracts green usually reduced and simpler than the leaves: flowers large, variable in color but calyx usually running from pink to pale rose-red: calyx tubular, in fruit elongating and tapering gradually from the distended base to the tip, becoming 4–5 cm. long and nearly equalling the corolla, cleft to the corolla lip before but only a few mm. behind, pubescence similar to that of the stems but shorter: corolla red-tipped but greenish or pale within the calyx, its tube 10–12 mm. long, the galea pubescent, 2–3 times as long as the tube, the lip short, scarcely more than 3 small teeth.

This fine species comes from near the Mexican border, having been secured in excellent condition by Mr. L. N. Goodding in Ramsey Canyon, Juachuca Mts., Arizona, Aug. 28, 1912, No. 1354. (Type, R. Mt.) It is a striking, easily recognizable plant by reason of its small dark leaves on pale slender stems topped by the relatively leafless inflorescences of large handsome pinkish-red flowers.

Hymenoxys virgata, n. sp.

Probably biennial but possibly perennial, the woody root having a single crown covered with imbricated leaf bases and (in this specimen) with no indication of former stems: stem 5–6 dm. high, solitary, simple, slender, erect, branched only into the simple corymbiform panicle, reddish-brown below, gradually paler upward: leaves inconspicuously punctate few and small, the largest only 3–5 cm. long; the lower pinnate or rather actually twice trifid, *i.e.* trifid with the middle lobe again trifid, the blade or divided portion shorter than the basal petiolar part, all the lobes narrowly linear, narrower than the petiole which is barely 1 mm. broad, upward becoming sparser and smaller, merely trifid and then passing into small linear bracts: pubescence (?) sparse, reduced to minute scattering white flattened scales on leaves and stem with denser patches at the nodes: heads few, 6–8 mm. high, 8–10 mm. broad: involucral bracts in 2 rows, low-carinate, obscurely scaly-fringed, 4–5 mm. long the outer oblong, sub-acute; the inner broader, obovate; obtuse or truncately 2–3 toothed: rays few, the ligule oblong, about 10 mm. long, spreading or reflexed: disk flowers 20–40, well surpassing the involucre; the tubular corollas slightly enlarged upward, the short teeth more or less granular-penicillate in appearance, pappus of few broad thin white scarious scales somewhat shorter than the corollas; achenes obscurely 4-angled, appressed-pubescent, 2–3 mm. long, nearly as long as the corolla.

This species is one more member of the section in which the outer bracts are free nearly to the base. *H. canescens* and *H. biennis* constitute one pair of the group of four and *H. Greenei* and *H. virgata* the other. This

latter pair may be contrasted as follows: *H. Greenei*, leafy and branched, stocky, only 3 dm. high; outer bracts tapering from the base; rays 3-lobed; pappus paleae ferruginous, tapering gradually into a long awn; pubescence on achenes fuscous. *H. virgata*, unbranched, the few leaves diminishing in size and number from the base up, 6 dm. or more high, slender and wand-like in aspect: outer bracts broader near the middle; rays merely toothed; pappus paleae broader upward and abruptly contracted to a short awn; pubescence on achene glistening white as are also the pappus paleae.

The type R.Mt. was secured by G. E. Osterhout, Grand Canyon, Arizona, on Bright Angel Trail, June 22, 1928. No. 6991.

Porophyllum putidum, n. sp.

Perennial from a low branched caudex which scarcely rises above the surface of the ground: stems very numerous, erect and fascicled, the dry pressed specimens very broom-like in appearance, about 3 dm. high: leaves filiform, quite inconspicuous among the slender rush-like stems, 1–3 or more cm. long, with a short dark-colored acumination that is apparently deciduous, the oil-glands few, mostly median, large enough to cause some distension in the leaf: the inflorescence of each stem a terminal corymb of few heads; the floral leaves not much reduced, as in the cauline, the margins revolute, thus accentuating the filiform appearance: heads cylindric-campanulate, about 20 mm. high; the glabrous purplish bracts 5, oblong-linear, abruptly subacute, scarcely 15 mm. long, oil glands mostly in three equidistant pairs; flowers 20 or more, tube proper slender, scarcely expanding in the long throat which bears ovate-lanceolate teeth half as long as the throat; tube proper equalling the throat and teeth; brown lines run from the top of the tube to the sinuses and then converge to form a brown border on the teeth; pappus fuscous, copious, equalling the corolla and exceeding the involucre by one-third its length; achenes slender, scarcely as long as the pappus, grayish with a short stiff pubescence, tapering but only slightly at both ends.

The name *P. scoparium* Gray suggests a plant of this aspect but representative specimens from Western Texas show a plant with a very different involucre and floral characters quite out of harmony with this. The plant here described is, however, of that alliance rather than of that which has long received the relatives of *P. gracilis* Benth.

The excellent specimens at hand were collected by Leslie N. Goodding, no. 2277 (Type), in the foothills east of Douglas, Arizona, May 28, 1907.

Porophyllum gracile Benth. Bot. Sulp. 29. 1844 came from Magdalena Bay. It is a plant with a main axis (a short trunk) bushy branched above and becoming 6 dm. or more high (up to 12 dm.). In its typical form it does not appear in the arid interior Southwest which seems to furnish a number of allied forms. Among these are several described by Dr. Greene (Leaflets 2: 154 and ff.) some of which are of somewhat similar aspect but two of them are characterized as herbaceous to the ground. These two can probably not be maintained as distinct species. The character "achenes pale straw-color" breaks down in that many specimens show dark and

light achenes in the same head (merely a matter of maturity). If one of them be reduced *P. Vaseyi* ranks *P. leucospermum*. The latter is included by Tidestrom in his Flora of Utah and Nevada but the description doubtless needs to be greatly liberalized.

Phlox Longifolia and Phlox Stansburyi and Their Immediate Relatives

There are two distinct alliances that have been inexcusably confused: viz., *P. longifolia* and its immediate relatives and *P. Stansburyi* and its immediate relatives. These are not only geographically fairly well dissociated but there are valid characters to correspond. With the delimiting of *P. Stansburyi*, as has been done in this paper, portions of that aggregate (heretofore designated by various names) have become detached. In the following key an attempt is made to show the relationship of these parts and, at least tentatively, designate their distribution. If even this much be accomplished, it will simplify their classification.

Key to Twelve Rocky Mountain Species of Phlox

Flowers erect.
- Not glandular, glabrous to lanate.
  - Plant pubescent throughout.
    - Leaves linear-subulate; minutely canescent..... 1. *P. austromontana*
    - Leaves linear-oblong; crisped-pubescent, mostly on the margins..... 2. *P. cortezana*
  - Plant glabrous, or pubescent above only.
    - Leaves definitely white-margined..... 3. *P. marginata*
    - Leaves not white-margined.
      - Leaves 3 mm. or more broad, 4–8 cm. long..... 4. *P. longifolia*
      - Leaves 2 mm. or less broad, 3–6 cm. long..... 5. *P. linearifolia*
- Glandular or viscid.
  - Plants glandular on pedicels and calyx only.
    - Leaves linear, 30–50 mm. long..... 6. *P. Stansburyi*
    - Leaves linear-oblong, 15–25 mm. long..... 7. *P. Woodhouseı oculata*
  - Plants glandular on stems and leaves also, at least above.
    - Stems and leaves erect.
      - Caudex branches slender, leaves narrow, cyme simple..... 8. *P. puberula*
      - Caudex branches woody, leaves broader, cyme subcapitate..... 9. *P. visenda*
    - Stems and leaves spreading.
      - Leaves linear-acerose, crowded throughout, glandular above only..... 10. *P. viridis*
      - Leaves broader and shorter, glandular-viscid throughout..... 11. *P. viscida*

Flowers nodding..... 12. *P. cernua*

The following general statements on distribution may serve as clues in the determination of material referable to this closely related group of twelve species of *Phlox*.

1. *P. austromontana* Coville, Contrib. Nat. Herb. 4: 151. 1893. In the middle altitudes of the drier mountains of the arid southwest. From southern Utah, to Nevada, Arizona and California. Distinguishing character: its uniform fine silvery canescence.
2. *P. cortezana.* In the southern half of western Colorado and in adjacent Utah on the foothills and high sage-brush plains. Distinguishing character: a crisped pubescence throughout, of variable flattened hairs.
3. *P. marginata* Brand, Monog. Polem. 65. 1907. Known from eastern Oregon and across the southern part of Idaho. Distinguishing character: the cartilaginous leaf-margins.
4. *P. longifolia* Nutt., Journ. Acad. Philad. 7: 41. 1834. On the Pacific slope of the Rocky Mountains in Wyoming, northern Utah, Idaho and Montana. Distinguishing character: glabrate herbage.
5. *P. linearifolia* Gray, Proc. Am. Acad. 8: 255. 1870. Nearly the same range as *P. longifolia* but farther northward and westward. Distinguishing character: filiform long usually glabrous leaves.
6. *P. Stansburyi* (Torr.) Heller, Bull. Torrey Club 24: 478. 1897. In the mountains of New Mex. on the Texan border (Organ Mts.). Distinguishing character: corolla-tube at least twice as long as the calyx; leaves linear.
7. *P. Woodhousei oculata.* White Mts., eastern Arizona. Corolla eyed, its tube never twice as long as the calyx; leaves narrowly oblong.
8. *P. puberula* (E. Nels.) A. Nels., Coult. and Nels. Man. 397. 1909. This well marked species is decidedly local; at least so far, it has been found only in the vicinity of Evanston, Wyoming. Distinguishing character: viscid-puberulence on the green erect herbage.
9. *P. visenda.* In the region of the Grand Canyon of Arizona and extending probably into the wooded table lands of adjacent Utah, Nevada and California. Distinguishing character: the subcapitate flower cluster crowning the erect foliose stems.
10. *P. viridis* E. Nels., W. No. Am. Phloxes, 24. 1899. Western Idaho, and eastern Oregon and Washington. Distinguishing character: the green crowded linear-aculeate leaves. *P. aculeata* A. Nels.
11. *P. viscida* E. Nels. l.c. Range not worked out but probably extending southward from Washington into California. Distinguishing character: viscid-villous throughout.
12. *P. cernua* E. Nels. l.c. Probably very local but well known from Montrose Co., Colorado. Distinguishing character: flowers nodding both in bud and in full anthesis.

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STUDIES IN THE CHYTRIDIALES V. A FURTHER STUDY OF SPECIES OF THE GENUS *ENTOPHLYCTIS*

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During the past three years since *E. heliomorpha* was first discovered and reported in cells of American Characeae the author has had this species under almost continuous observation. Dying and decaying old internodal cells of the Characeae are veritable storehouses of starch grains and other food substances and make an excellent medium for the growth of chytrids, so that by maintaining a few battery jars of cultures of these plants it is possible to have a number of species on hand almost constantly for study. Species of *Diplophlyctis* and *Entophlyctis* are unusually easy to maintain in this manner, and since they are comparatively large in size they lend themselves readily to morphological studies. They generally occur in great profusion, so that single host cells which are rich in food material may harbor hundreds of individual thalli in almost every stage of development.

Entophlyctis heliomorpha has been kept under observation primarily to determine its host range and the method of germination of the resting spores. Dangeard (1886–1888) reports its occurrence in species of *Nitella*, *Chara*, and *Vaucheria*, and I (1928) have found it in a large number of stoneworts, including *Chara fragilis*, *C. delicatula*, *C. coronata*, *C. zeylanica*, *C. disjuncta*, *Nitella flexilis*, *N. gracilis*, *N. tenuissima*, and *N. glomerulifera*. Although these forms appeared identical morphologically on the various hosts it was not certain whether they were the same species of biological races confined to a particular host. Cross inoculation experiments were thus begun with all available species of Characeae and various other algae. Dying and dead but sterile internodes of *Nitella* and *Chara* and filaments of *Spirogyra*, *Vaucheria*, *Oedogonium*, *Mougeotia*, and *Hydrodictyon* were placed in the jars containing the infected Characeae cultures. The results obtained were negative. The data, however, are not sufficient and conclusive enough to warrant the assumption that these are physiological species. Hence, more extensive inoculation experiments were begun and are now under way.

During the progress of these experiments with *E. heliomorpha* cultures of *Cladophora* which had been brought into the laboratory in the fall of 1930 and grown apart from the infected Characeae became profusely parasitized with a species of *Entophlyctis*. Previous to this time I had also found some extremely large thalli of *Entophlyctis* in cells of *N. flexilis* and *Elodea* sp., which because of their unusual size seemed at first to be new species. It remains to be seen, however, whether or not they

really are new species; but I shall, none the less, describe them. At the present time there are listed in the literature approximately ten species of *Entophlyctis*, all but two of which occur in cells of algae. Few of these species have very definite morphological characters, outside of their size and host relationships, and a very large majority of them are very little known. Moreover, their range of variation under various conditions and in different hosts has been little studied. For these reasons it is very important that careful measurements and drawings be made of *Entophlyctis* species wherever they occur for comparison with the known species.

Entophlyctis Cienkowskiana or E. Vaucheriae

Inasmuch as Cienkowski (1857) and Zopf (1885) had described and figured *E. Confervae glomeratae* and *E. Cienkowskiana* in cells of *Cladophora* it was assumed that the form which I had discovered in the same host was one of these species; and since neither of them has previously been reported from America, a study of its life history and morphology was begun for comparison with the European species. *E. Confervae glomeratae* and *E. Cienkowskiana* occur in the same host and appear identical morphologically; hence Zopf (1885) and Fischer (1892) list them as one species under the name *E. Cienkowskiana*. In the present paper I shall follow this terminology.

The general morphology and life history of the species which I have observed in cells of *Cladophora* sp. is shown in Plate XXXV. In method of development it is essentially the same as described for *E. Cienkowskiana* by Zopf and Cienkowski, but in certain almost indescribable respects it looks different. Figure 1 shows a short young *Cladophora* cell with sixteen chytrid thalli in various stages of development. The number of parasites in a single cell varies considerably with the size of the host cells and the degree of parasitism. Zopf (Pl. 6, fig. 14) shows a cell of *Cladophora* with more than thirty thalli. When first discovered the cell which is shown in my figure 1 was partly dead although remnants of the reticulated chloroplast were still quite distinct and easily recognizable. Starch grains are present in great numbers, and many of them lie grouped together around the pyrenoids. It is not apparent whether the pathological condition of the host is due to the presence of the chytrid, or that it has made possible the entrance of the parasite. All of the infected *Cladophora* cells were more or less in the same condition, and since the culture had been growing in small battery jars under probably unfavorable conditions it is quite possible that they were in a physiological and pathological state which rendered them quite susceptible. At the present time I am inclined to the view that the chytrid gains entrance only after the host has reached a certain pathological condition, for the reason that I have so far been unable to find early infection stages of healthy cells and trace the inception and progress of the disease. Zopf and Fischer likewise report that *E. Cienkowskiana* is to

be generally found only in dying or dead host cells, and I have observed the same conditions in *E. heliomorpha* in cells of Characeae. It must be borne in mind, however, that the presence of the deep green chlorophyll of living *Cladophora* and stonewort cells may readily obscure the initial infection stages and the hyaline zoösporangia and rhizoids, and that they do not become readily visible until the disease has reached a certain stage.

The epidemiology of Chytridiacean parasites on algae is little known and understood. The conditions necessary for their appearance, growth and development, and parasitism have been little studied. My observations up to the present time on their occurrence in nature and laboratory cultures of algae is that with the exception of *Entophlyctis* and *Diplophlyctis* they appear rather suddenly and run their course quickly. During the climax of an epidemic I have attempted to simulate conditions which seemed to be optimum for the continuation of the disease, but the results have been largely negative. In the case of *E. Cienkowskiana* I have repeatedly added filaments of *Cladophora* from out-of-doors which were dead or dying to the infected laboratory cultures, and, while a few of the cells became infected, I was, none the less, unable to maintain the epidemic. Similar experiments with mechanically injured and thermally killed host cells have been unsuccessful. Doubtless, variations in the degree of virulence of the parasite as well as the susceptibility of the host at different times play just as significant a rôle in an epidemic as the external environment conditions.

The walls of the *Cladophora* host cell shown in Plate XXXV, figure 1, are quite thick, and at several places the globular portion of the chytrid thallus has developed between the layers and spread them apart in the same manner that *Diplophlyctis intestina* (Karling 1928*b*) does in cells of *Chara* and *Nitella*. In such cases the rudiments of the incipient sporangium begins to develop within the host cell wall instead of in the lumen of the cell, and as it enlarges the layers are separated and spread apart, as is indicated by the minute thalli at *a* and *b*. The rhizoidal systems, however, extend for a considerable area within the cell.

A number of early infection stages are also shown in this figure. At *a*, *b*, *c*, *d*, *e*, and *f* the spore cases and penetration tubes are still present and their relation to the developing thallus is well illustrated. In a previous study (1928*a*) of the early stages of zoöspore germination and thallus development of *E. heliomorpha* I have described the origin of the rhizoidal system as being comparatively late in relation to the incipient zoösporangium. From this study of *E. Cienkowskiana* or *E. Vaucheriae* it seems that as a general rule the rudiments of the rhizoids are formed somewhat earlier. In figure 2 is shown a germinating zoöspore whose penetration tube has branched to form two minute rhizoids. There is no evidence, however, of the incipient zoösporangium as yet. It develops subsequently, as is suggested in figures 1*a*, 1*b*, 3 and 4, Plate XXXV, just back of the point of

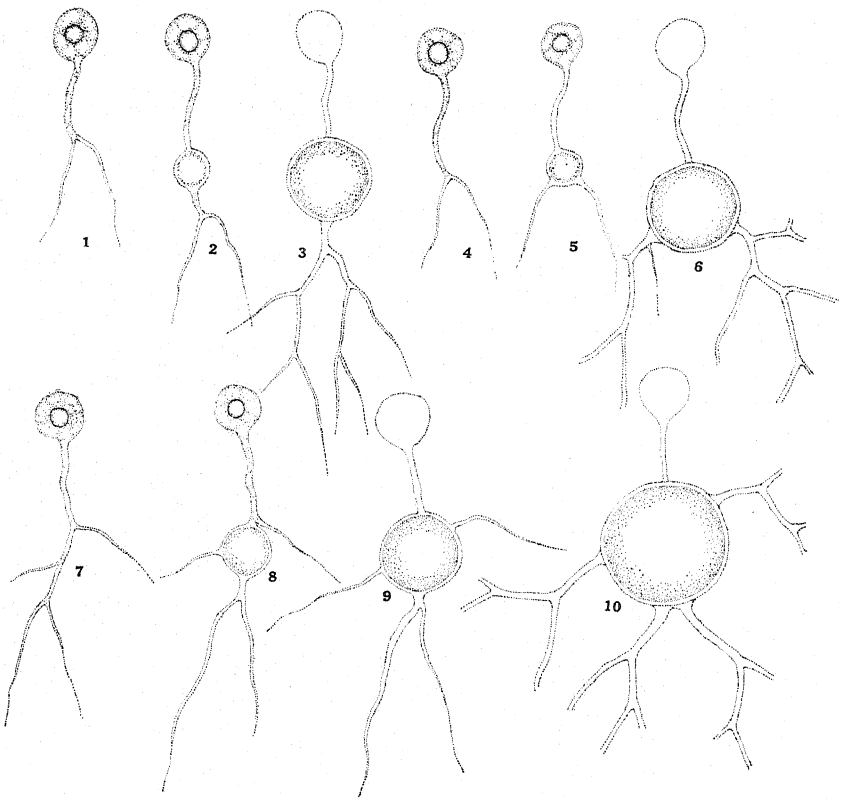
origin of the rhizoids. The time of its origin relative to the rhizoids is very similar to that of the apophysis of *Diplophlyctis intestina*.

The subsequent stages of development of the zoösporangium, resting spores, and cleavage are shown in figures 5 to 9. They are so similar in general to the description and figures of Zopf that further discussion of them would doubtless be superfluous. The morphological appearance of the mature thallus however, seems different in many respects, and merits in my opinion special attention, particularly since these differences have been the basis of species distinction. It is obvious from the thalli shown in Plate XXXV that the rhizoids do not arise all over the surface of the zoösporangium and resting spore but are more or less confined to a particular region, generally opposite the penetration tube and spore case. The number may vary from 1 to 4, but in the majority of the thalli which I have observed they are usually fairly close together. The majority of zoösporangium and resting spores of *E. Cienkowskiana* figured by Zopf have several rhizoids which arise and extend in a radial fashion, although monorhizoidal thalli are not lacking. Similarly, polyrhizoidal thalli are predominant in *E. heliomorpha*, with occasional monorhizoidal ones.

Wildeman (1896) used the number of rhizoids and their localization at the base of the zoösporangium as the distinguishing species characters of *E. Characearum* in oögonia of *Chara*. Petersen (1910) likewise recognized these characters as being morphologically significant and restricts the genus *Entophlyctis* to forms whose rhizoids arise at various points. In view of the occasional presence of similar monorhizoidal thalli in *E. Cienkowskiana* and *E. heliomorpha* I have (1928*a*) questioned the validity of Wildeman's species and the restrictions of Petersen. However, since observing the predominence of thalli with one or two rhizoids localized at the base of the zoösporangium or resting spore in the species on *Cladophora* I have reconsidered the possibilities that these might be specific characters.

The number and relative position of rhizoids in the mature thallus depends on two conditions: First, the manner and number of times the penetration tube branches; and second, the point of origin of the incipient zoösporangium or resting spore relative to these branches. In text figures 1 to 10 I have attempted to illustrate and analyze some of the ways in which mono- and polyrhizoidal thalli may arise. This analysis is based on the assumption, for which I have already presented some evidence, that the rudiments of the rhizoidal system are laid down before the incipient zoösporangium. Text figure 1 shows a germinated spore whose penetration tube has branched to form two small rhizoid rudiments. If no more branching occurs and the incipient zoösporangium begins to develop, as in text figure 2, above these branches a monorhizoidal thallus (text fig. 3), will be formed. On the other hand, if it begins to develop at the juncture of the two branches as in text figure 5, they will gradually be carried apart and eventually come to lie in almost opposite positions on the surface of

the zoösporangium as is shown in text figure 6. We will next consider a case in which three or more rhizoid rudiments are present. Such a young thallus is shown in text figure 7. If then as is shown in text figure 8 the sporangium begins to develop at a point more or less central to the rhizoids they will gradually come to occupy the positions in text figures 9 and 10, and thus a thallus with several rhizoids at various points will be formed.



TEXT FIGS. 1–10. Hypothetical thalli of *Entophlyctis* demonstrating some of the possible ways by which the number and position of the rhizoids relative to the zoösporangium or resting spore may vary.

From such hypothetical cases it is possible to postulate the origin and occurrence of various types of thalli, but as noted before, the entire analysis rests on the assumption that the incipient zoösporangium begins to form subsequent to the rudiments of the rhizoids. As to whether or not the number and localization of the rhizoids are definite species characters as Wildeman and Petersen claim is difficult to determine. Careful statistical studies of many successive generations as well as cross inoculation experiments may throw some light on the problem.

Another contrasting difference to Zopf's figures of *E. Cienkowskiana*

which I have observed in this species is the presence of a large vacuole in the developing zoösporangia. They were so universally present and similar to those figured by Fisch (1884) for *E. Vaucheriae* that I believed at once I had discovered this species in *Cladophora*. Vacuoles are such transient characters, however, that it is imprudent to base any definite distinction on their presence. But the majority of thalli of this *E. Vaucheriae* also have a limited number of rhizoids localized at the base of the zoösporangium and resting spore, and for these reasons the species which I have studied is more similar to it than to *E. Cienkowskiana*. This presents the problem as whether or not they may be the same species. Fischer reports *E. Vaucheriae* from *Vaucheria sessilis* and *Spirogyra* sp. It is not improbable that it might go to *Cladophora*, or *E. Cienkowskiana* might go to *Vaucheria*. According to Dangeard (1888) *E. heliomorpha* also occurs on *Vaucheria*. I have made many attempts to infect filaments of this alga with the chytrid species from *Cladophora* without any success. Until further experiments of this nature have been carried out the problem of identity of species must remain in the present state. It is obvious that my study has complicated the situation more than clarified it, but it is only by facing such probabilities as have been presented, it seems to me, that we will make real progress.

Giant Thalli of Entophlyctis

In 1928 I described some unusually large zoösporangia and resting spores of *E. heliomorpha* in cells of *N. glomerulifera*, and in the spring of the following year I found numerous ones in internodes of *N. flexilis* that were even larger. Their enormous size in contrast to the average *E. heliomorpha* fruiting structures attracted my attention at once, and I began a careful study of their occurrence, life history, and general morphology and measured the zoösporangia, zoöspores, and resting spores to determine whether or not this might be a giant species of *Entophlyctis*. Since that time I have discovered giant forms in cells of *Cladophora* and *Elodea* sp., which indicates that they are of rather wide occurrence. In Plates XXXVI–XXXVIII are shown their habit of growth and general morphology in cells of *N. flexilis*, *Elodea* sp., and *Cladophora* sp., respectively. Their life history and method of development are so similar to those of other species of *Entophlyctis* that it will be unnecessary to discuss these phases in detail.

I shall consider first the form which occurs in internodes of *N. flexilis*. In table 1 are shown the size of the zoösporangia, zoöspores, resting spores, number of rhizoids, and their location with respect to the rest of the thallus. The spherical zoösporangia vary from 22 to 53 μ in diameter, with an average of 35 μ from measurements of a hundred. The smallest zoösporangia thus fall within the range of the largest ones described for *E. heliomorpha* in 1928 from cells of *N. glomerulifera*. The number of rhizoids varies from 1 to 7, but more than one is the general condition. Quite often the rhizoids

arise close together in pairs or threes as is shown in figures 10 and 11, Plate XXXVI. They may be localized at the base or distributed at almost any point on the surface of the zoösporangium. In this respect they are very similar to *E. heliomorpha* which also occurs in *N. flexilis*. In figure 15, Plate XXXVI, is shown a large zoösporangium whose neck has branched before penetrating the wall of the host cell. The two branches extend more or less parallel along the surface of the *Nitella* cell for a distance of 160 μ.

The zoöspores, on the other hand, are of approximately the same size as those of other species of *Entophlyctis*. I have observed only a few zoösporangia with escaping zoöspores, and hence there have been few opportunities of measuring the spores. From a study of twenty I have found them to vary from 4 to 7 μ. In appearance, habit of swimming, and amoeboid motion they are essentially similar to those of other species. Figures 12 and 13 show their general structure and method of germination.

The resting spore are unusually large, varying from 26 to 80 μ in diameter, figures 10, 11 and 14, Plate XXXVI. The smallest of these are thus greater in size than the largest resting spores of any previously described species. The contents of the resting spore is finely or coarsely granular with a large number of globules of variable size. The wall is often as much as 3 μ thick and sometimes almost hyaline to light brown and olivaceous in color. The number of rhizoids vary from 1 to 7 and may be localized at the base or distributed at various intervals on the periphery of the spore. At their point of origin they are usually fairly large in diameter and appear quite bristle like. The system as a whole, however, is not as richly branched as in *Diplophlyctis intestina*.

TABLE 1. *Variations in the Sizes of the Zoösporangia, Zoöspores, and Resting Spores, and the Number and Distribution of the Rhizoids of Giant Thalli of Entophlyctis*

Host	Size of Zoösporangia	Size of Zoöspores	Size of Resting Spores	No. of Rhizoids and their Distribution
Nitella flexilis.	22–53 μ average 35 μ	4–7 μ	26–80 μ average 45 μ	1 to 7. May be localized at the base or distributed at various points on the surface of the resting spore.
Elodea sp.	20–45 μ average 37 μ	4–7 μ	21–46 μ average 30 μ	1 to 3. Usually localized at the base of the resting spore.
Cladophora sp.	25–56 μ average 36 μ	4–7 μ	—	1 to 3. Usually localized at the base of the resting spore.

The giant form on *Elodea* sp. was discovered only after the host plant was dead and the leaves were in an advanced stage of decay. Repeated examination of living leaves from healthy plants in the same jar failed to show their presence, so that the organism appears to be a sapro-

phyte. In Plate XXXVII I have shown the essential features of the life history and general morphology of the organism as it occurs in cells of *Elodea*. The zoösporangia show an extreme variation in size, shape and number of necks. The conditions here are very similar to those described for *D. intestina* (1930) in *Nitella* cells which are rich in food. Figures 18, 19, 20 and 21, Plate XXXVII, show the variations in size and shape. In size the spherical ones are 26 to 45 μ in diameter, with an average of 34 μ. One neck per sporangium is the type number, but as many as six have been found on a single zoösporangium. They may be from 3 to 10 μ in diameter and extend in the extreme cases to a distance of 200 μ. The rhizoids may be 1 to 4 in number, but they are usually close together and in the majority of cases arise from the base of sporangium.

The zoöspores are shown in figure 25, Plate XXXVII. With respect to their cilia, refractive spot, and method of swimming they are typical of the family Rhizidiaceae. Their method of germination and the stages in the formation of the young thalli are shown in figures 26, 27, and 28.

The resting spores, figures 22, 23, and 24, Plate XXXVII, are predominantly spherical in shape, but quite frequently elongated, oval ones may be found which have in general the same shape as the host cell. As is shown in table 1 the spherical resting spores vary from 20 to 45 μ in diameter with an average of 30 μ from a measurement of one hundred. The content is very characteristic and makes the spores easily visible in the host cells. In the center of the spherical spores is a very large vacuole or globule, while the elongated and oval ones may have two or three. The remainder of the protoplasm is very coarsely granular and presents the appearance as if it had undergone cleavage. The segments, however, are smaller than the ordinary zoöspores and quite angular in many cases. In living material the wall of the resting spore is hyaline to greenish brown and olive green in color, quite thick, 2 to 4 μ, and very resistant to pressure.

I have already discussed the rhizoids briefly in connection with the zoösporangium. The same general conditions hold with respect to the resting spores, except that here the walls of the rhizoids often appear much heavier and slightly olive green in color, which is doubtless due to age. They may be as much as 5 μ in diameter at the point where they leave the surface of the resting spore.

The giant form in *Cladophora* appeared at the same time as *E. Cienkowskiana* or *E. Vaucheriae* described above, and the two were frequently found side by side in the same cell. Figure 29, Plate XXXVIII, shows the comparative size of the two thalli in a short cell of *Cladophora*. The sporangium and rhizoidal system of the giant form in this particular instance is more than double that of *E. Cienkowskiana*. As is shown in table 1 the sporangia vary from 25 to 56 μ, with an average of 36 μ. The rhizoids may be 1 to 3 in number, but as in the case of the form described above from *Cladophora* they are usually localized at the base.

They are generally rather large in diameter, especially at the point where they leave the surface of the zoösporangium. I have found numerous ones which were as much as 5 μ in diameter in this region. The habit of branching and general appearance is often quite characteristic. The rhizoids have a tendency to branch dichotomously, but since the branches do not always grow to equal size and dimensions a more or less zigzag axis is maintained, as is shown in figure 30, Plate XXXVIII. This characteristic is well illustrated by Zopf's drawings of *E. Cienkowskiana* also. It gives the rhizoidal system as a whole a somewhat bristle-like, rigid appearance, so that with a fair amount of practice a student of this genus soon comes to recognize the thallus with great readiness.

As to the zoöspores, they are strikingly similar to those of the giant form in *N. flexilis*, as far as my observations go at the present time. Figures 31 to 36, Plate XXXVIII, show a sporangium after the completion of cleavage, escape of the zoöspores, their general appearance, stages in germination, and young thalli. Giant resting spores have not yet been observed in *Cladophora*, so that it is impossible to compare their size and morphology with the others.

Identity of the Giant Thalli

As to the identity of the giant thalli which I have described above there are several possibilities which I shall discuss in detail. The probability that they are monstrosities of *E. heliomorpha*, *E. Cienkowskiana*, or *E. Vaucheriae* is supported to a certain extent by the fact that they have always been found associated with these species and have a similar life history. If such proves to be the case these three species will have extreme ranges of variation in size. In *E. heliomorpha*, for instance, the resting spores would then vary from 8 to 80 μ in diameter. However, the coarseness of the rhizoids and thickness of the resting spore wall as well as the general appearance of the giant thalli in comparison with the average thalli of these three species makes one seriously question this possibility.

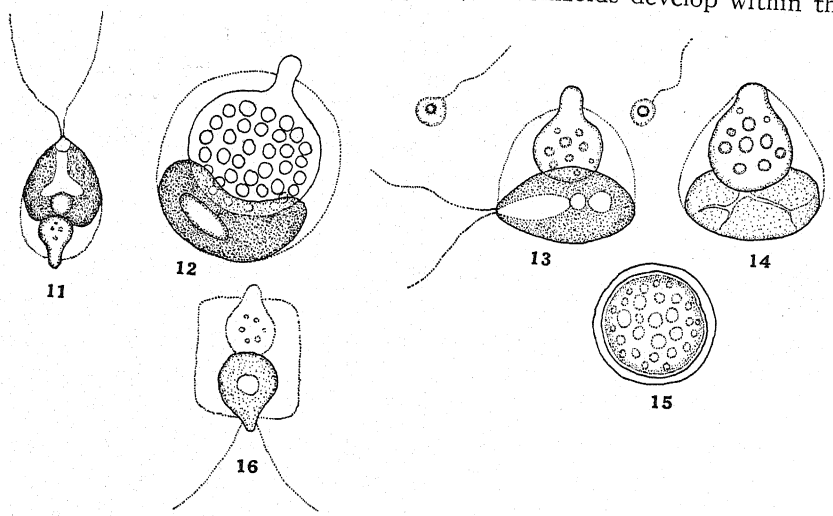
Species with thick and coarse rhizoids, however, have been reported before. In *E. bulligera* which Zopf (1885) figures and describes as a new giant species from cells of *Spirogyra* the rhizoids have a diameter approximately one-fourth that of the zoösporangium (Pl. 7, fig. 6), and extend for a distance of 150 μ. They may be few in number and localized at the base of the zoösporangium or as many as five distributed at various points. As in the case of the giant form which I have described from *Elodea* the rhizoids are able to penetrate and spread in neighboring host cells. *E. Salicorniae* (Nemec, 1912) likewise has a coarse, thick-walled and widely distributed rhizoidal system. The chance that the giant forms shown in Plates XXXVI, XXXVII, and XXXVIII might be *E. bulligera* seems improbable if the method of zoöspore germination described by Zopf is correct. In this species the spore case does not disintegrate after germination, but remains attached to the developing zoösporangium and eventually becomes a short

sporangial neck or papilla. I have so far failed to find any evidence of this in the giant thalli which I have studied.

There remains finally the possibility that they are new giant species. In view of the great diversity in form and size which thalli of *Entophlyctis* frequently show it seems premature at the present state of my knowledge to establish new species for these forms. Before their identity is definitely established it will be necessary to isolate and grow single sporangia or resting spore cultures and study their variation for a number of generations as well as to carry out extensive cross inoculation experiments. Until further conclusive evidence as to their identity is at hand I shall consider them as uncertain species.

General Résumé of the Genus Entophlyctis

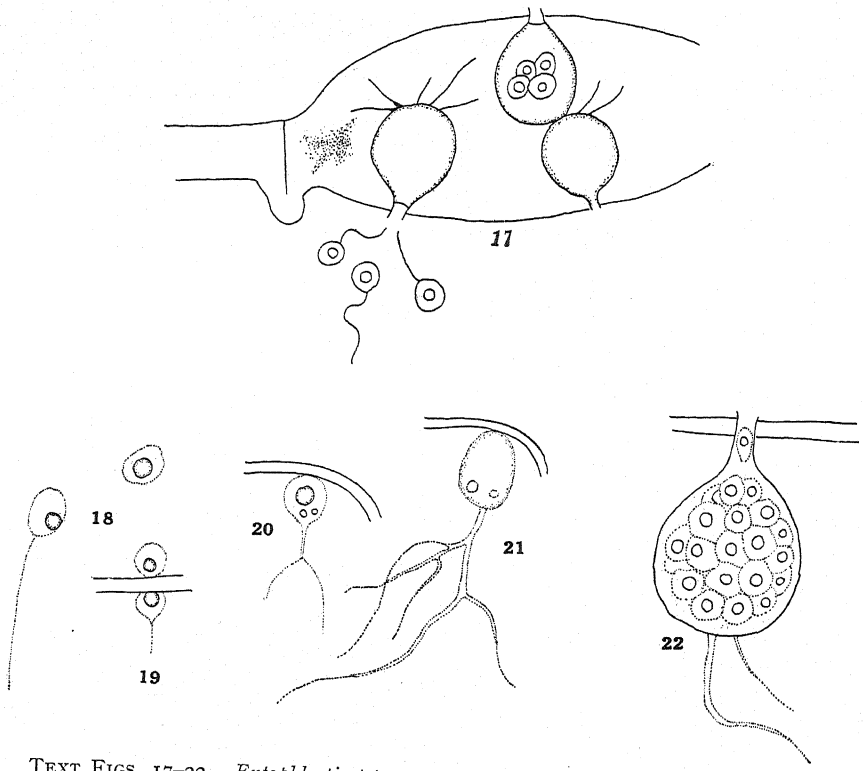
The genus *Entophlyctis* was established by Fischer in 1892 as a member of the Sporochytriaceae, into which family he had combined the Rhizidiaceae and Polyphagaceae. At first the genus included all *Rhizidium* species whose entire thalli, zoösporangia, resting spores, and rhizoids develop within the



Text Figs. 11–16. *Entophlyctis apiculata*. Fig. 11. A swarmspore of *Gleococcus mucosus* parasitized by a young thallus. Fig. 12. A later stage showing the mature zoösporangium with zoöspores, after Braun (1855). Fig. 13. A swarmspore of *Gloeococcus mucosus* (?) infected with *E. apiculata*. Fig. 14. A mature thallus with a minute rhizoidal system. Fig. 15. A resting spore, after Zopf (1885). Fig. 16. A swarmspore of *Phacotus viridis* parasitized by an indeterminate chytrid species, after Dangeard (1889).

host cell, but in 1897 Schröter limited it to the intracellular or endophytic species whose zoösporangia lack an apophysis (Wurzelblase) at the base. According to Schröter's limitations of the genus there are listed in the literature at the present time approximately ten species, all but two of

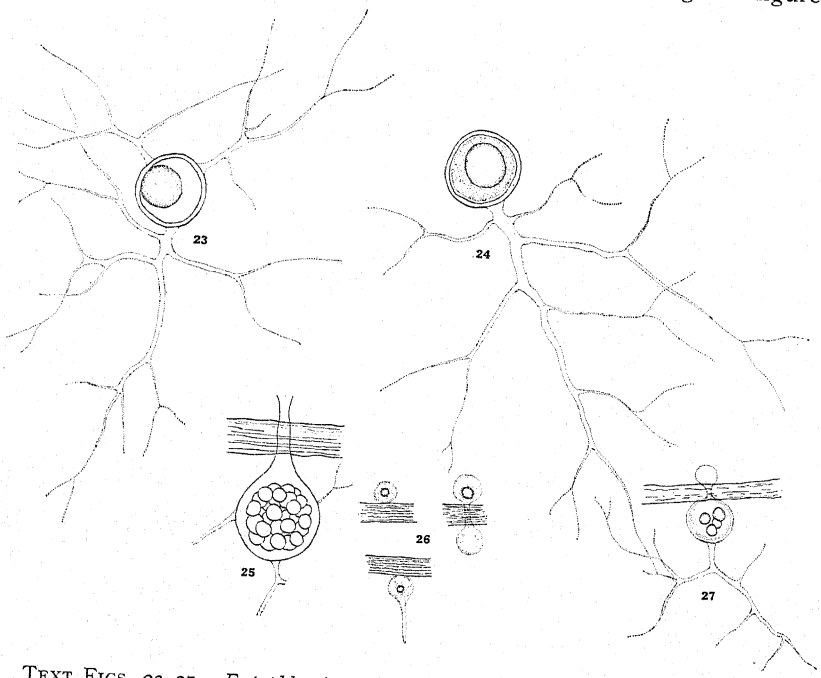
which occur in cells of algae. In table 2 I have presented in tabular form the size of the zoösporangia, zoöspores, and resting spores, the hosts, the number and distribution of the rhizoids relative to the resting spore, and the character of the spore wall of these species. Whether or not all of these are legitimate species remains to be determined. I shall discuss them briefly in the order of their discovery.



TEXT FIGS. 17–22. *Entophlyctis tetrasporum* and *E. Confervae glomeratae*. FIG. 17. A cell of *Rhynchonema* with three zoösporangia of *E. tetrasporum*, after Sorokine (1883, 1889). FIG. 18. Zoöspores of *E. Confervae glomeratae*. FIG. 19. An early infection stage. FIGS. 20 AND 21. Young thalli in cells of *Conferva glomerata*. FIG. 22. A mature zoösporangium with zoöspores, after Cienkowski (1857).

Entophlyctis apiculata was first described and figured by Braun in 1855 as *Chytridium apiculatum* in swarmspores of *Gloeococcus mucosus*. He describes the zoösporangia as being from 11 to 13 μ in diameter, but failed to observe the presence of rhizoids. It is, therefore, impossible to determine from Braun's figures alone whether or not he was observing a species of *Entophlyctis*, but in 1885 Zopf discovered a similar form on the same host which he named *Rhizidium apiculatum*. His description and figures are identical with those of Braun with the exception that in host cells whose

chlorophyll had been extracted with alcohol rhizoids are visible. It is thus obvious from Zopf's observations that Braun had studied the same species but overlooked the rhizoids. In text figures 11 to 15 are shown the thalli as they have been figured by Braun and Zopf. In addition Zopf discovered the small smooth-walled resting spores which are very similar to those of the other species of this genus. In 1889 Dangeard figured a



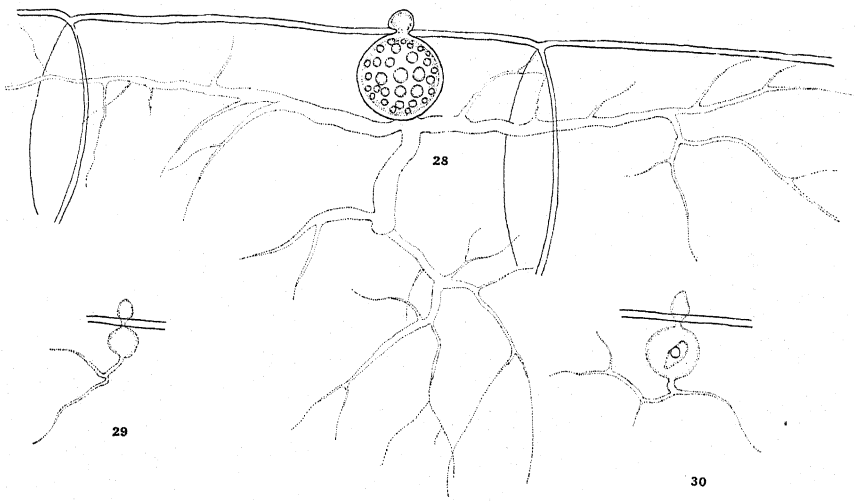
TEXT FIGS. 23–27. *Entophlyctis Cienkowskiana*. FIGS. 23 AND 24. Mono- and polyrhizoidal resting spores. FIG. 25. A mature zoösporangium with zoöspores. FIG. 26. Infection stages. FIG. 27. A dwarf monorhizoidal thallus with four zoöspores, after Zopf (1885).

chytrid on *Phacotus viridis* which he regarded as an intermedial *Chytridium* species. As is shown in text figure 16, his drawing is almost identical with those of Braun and Zopf, and this is, doubtless, a similar species of *Entophlyctis* in which he has overlooked the rhizoids.

The second species, *E. Confervae glomeratae*, was discovered shortly afterwards in 1857 by Cienkowski in cells of *Conferva glomerata*. Following Braun's creation and distinction of the *Rhizidium* the previous year (1856), he placed it in this genus. Inasmuch as Cienkowski's species appear identical with *E. Cienkowskiana* which was described by Zopf from the same host in 1884, I shall treat the two as the same species. Sorokine (1883, 1889) and Zopf likewise placed this species in the genus *Rhizidium*, and it remained there until incorporated in *Entophlyctis* by Fischer in 1892.

Schröter (1897) retains it in this genus, with the restrictions already described. In the text figures 18 to 27 I have shown the zoöspores, infection stages, zoösporangia, and resting spores as they are figured by Cienkowski and Zopf. The descriptions of Sorokine are very fragmentary, but Zopf gives the complete life history with the exception of the germination of the resting spores. I have already discussed the variations in size of the zoösporangia and resting spores and the number and distribution of the rhizoids, and since these characters are concretely presented in table 2, it is unnecessary to deal with them further.

Entophlyctis tetrasporum is a minute species which Sorokine (1883,



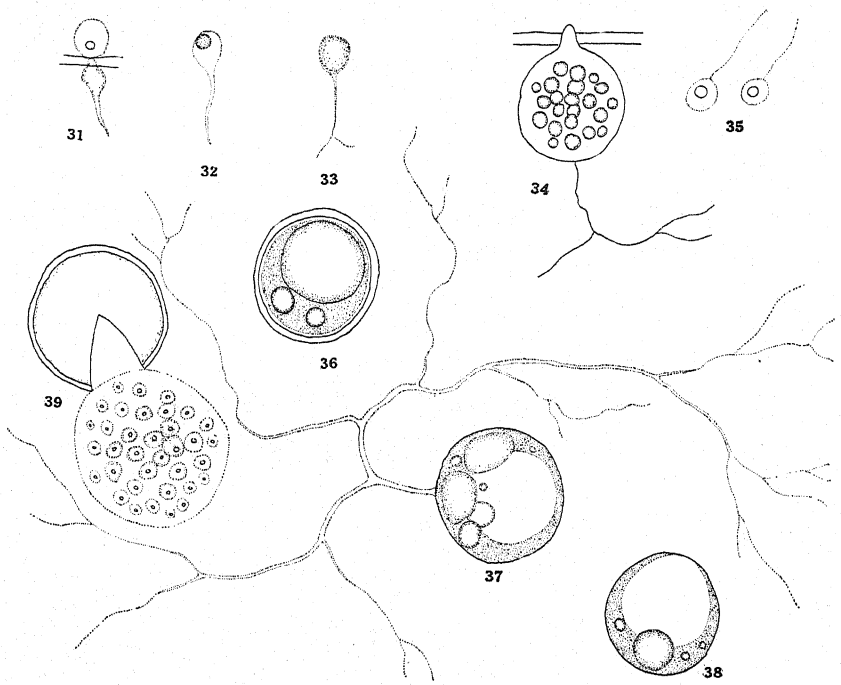
TEXT FIGS. 28–30. *Entophlyctis bulligera.* FIG. 28. A mature thallus extending into three cells of *Spirogyra crassa.* FIGS. 29 AND 30. Young thalli, after Zopf (1885).

1889) found in filaments of *Rhynchonema.* The zoösporangia are very small and, according to Sorokine, bear only four zoöspores. His figures, one of which I have shown in text figure 17, are so small and poorly made that they are of little value in distinguishing the species. The small size of the zoösporangia and reduced number of zoöspores are questionable species characters, it seems to me. In *E. Cienkowskiana* Zopf shows (Pl. 7, fig. 4) sporangia with only four, and in *E. heliomorpha* I have found (1928, Pl. L, fig. 13) some with but two zoöspores. These are dwarf zoösporangia which occur only occasionally, and it is perhaps probable that Sorokine's *E. tetrasporum* is a dwarf form of another species. He does not describe resting spores, so that further identification on this basis is impossible.

I have already considered *E. bulligera* in connection with the giant thalli described above. This species is not only characterized by its large size but by the unusual behavior of the zoöspores after germination (text figs. 28–30). According to Zopf, the zoöspore case and penetration tube do not

disintegrate after termination, as has been described for the other species of *Entophlyctis*, but remain as a globule attached to the zoösporangium. Then at maturity they serve as a sporangial neck and papilla for the escape of the zoöspores. If Zopf's description is correct the generic distinction of *Entophlyctis* as being wholly endophytic is not as sharp and fast as Fischer supposed. Resting spores are unknown in this species.

As has been noted before *Entophlyctis Vaucheriae* and *E. Spirogyrae* were reported by Fisch in 1884 in cells of *Vaucheria sessilis* and *Spirogyra* sp.



TEXT FIGS. 31–39. *Entophlyctis Vaucheriae*. FIGS. 31–33. Infection stages and young thalli. FIGS. 34 AND 35. A mature zoösporangium and zoöspores. FIG. 36. A resting spore. FIGS. 37 AND 38. Vacuolated zoösporangia. FIG. 39. A germinating resting spore, after Fisch (1884).

Although he believes that the two species may be identical, Fisch nonetheless designates them by different names. He gives no description or figures of *E. Spirogyrae*, so that any diagnosis of this species is impossible. I have listed it separately in table 2 merely to call attention to its existence in Fisch's contribution. The vacuolated condition of the young zoösporangium and the predominence of one and two rhizoids located at or near the base have already been considered in relation to *E. Cienkowskiana* and are well illustrated in text figures 31 to 39. This species is of particular interest because of the claim that secondary sporangia develop from the

TABLE 2. *Size of Zoösporangia, Zoöspores, and Resting Spores, Hosts, Number and Distribution of the Rhizoids, and Character of the Spore Wall of Species of Entophlyctis*

Author	Species	Host	Zoösporangia	Zoöspores	Resting Spores	Number and Distribution of Rhizoids	Wall of Resting Spore
Braun 1855	*E. apiculata*	*Gloeococcus mucosus*	11.1 to 13.3 μ	—	—		—
Zopf	*E. apiculata*	*Glocococcus mucosus* (?)				1 to 2, at the base of the zoösporangium	Smooth
Fischer 1892	*E. apiculata*	*Gloeococcus mucosus* (?)					Smooth
Schröter 1897		*Gloeococcus*	11–13 μ 11–13 μ	Very small	—		Smooth
Cienkowski 1857	*E. Confervae glomeratae*	*Conferva glomerata*	10–23 μ	3–5 μ	5–25 μ	2 to 3, at the base or various places on the resting spore	Smooth
Sorokine 1883–1889		*Conferva glomerata*				3, base of the zoösporangia	—
Fischer 1892	*E. Cienkowskiana*	*Cladophora* sp.	5–25 μ	3–5 μ	5–25 μ		Smooth
Sorokine 1883–1889	*E. tetrasporum*	*Rhynchonema*	Very small	Very small, only four in a sporangium	—	2 to 5, at the base of the zoösporangium	—
Fischer 1892	*E. tetrasporum*	*Spirogyra*	Very small				—
Zopf 1884	*E. Cienkowskiana*	*Cladophora*	5–25 μ	3–5 μ	5–25 μ	1 to 6, at the base or various points on the surface of the resting spore	Smooth
Fischer 1892	*E. Cienkowskiana*	*Cladophora*	5–25 μ	3–5 μ	5–25 μ	—	Smooth
Schröter 1897	*E. Cienkowskiana*	*Cladophora*	5–25 μ	3–5 μ	5–25 μ	—	Smooth
Zopf 1884	*E. bulligera*	*Spirogyra crassa*	—	—	Unknown	1 to 5, at the base or various places on the surface of the zoösporangium	—
Fischer 1892	*E. bulligera*	*Spirogyra crassa*	—	—	—	—	—
Schröter 1897	*E. bulligera*	*Spirogyra crassa*	25 μ	—	—	—	—
Fisch 1884	*E. Vaucheriae*	*Vaucheria sessilis*	—	—	—	1 to 2, usually at the base of the zoösporangium	Smooth

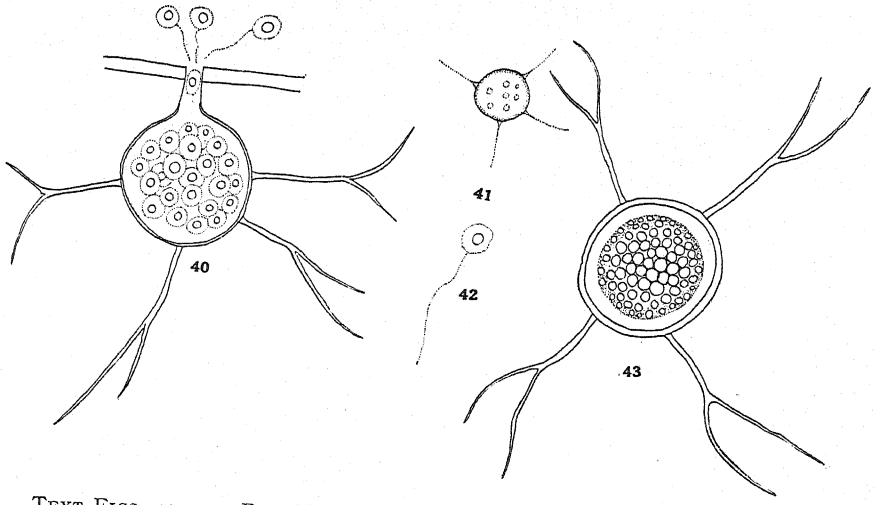
TABLE 2.—*Continued*

Author	Species	Host	Zoösporangia	Zoöspores	Resting Spores	Number and Distribution of Rhizoids	Wall of Resting Spore
Fischer 1892	*E. Vaucheriae*	*Vaucheria*	—	—	—	—	Smooth
Fisch 1884	*E. Spirogyrae*	*Spirogyra*	—	—	—	1 to 2, usually at the base of the resting spore	Smooth
Fischer 1892	*E. Spirogyrae*	*Spirogyra* sp.	—	—	—	—	Smooth
Dangeard 1886	*E. heliomorpha*	*Nitella, Vaucheria, Chara polyacantha*	10–20 μ	3 μ	Unknown	—	—
Dangeard 1886	*E. heliomorpha*	*N. tenuissima, Vaucheria, Chara*	10–20 μ	3 μ	Approx. same size as zoösporangia	6 to 7, at various points on the surface of the resting spore	Smooth
Fischer 1892	*E. heliomorpha*	*Nitella, Chara, Vaucheria*	10–20 μ	3 μ	—	6 to 7, at various places on the surface of the resting spore	Smooth
Karling 1928	*E. heliomorpha*	5 species of *Chara* 4 species of *Nitella*	8–30 μ	3–4 μ	8–24 μ	2 to 7, at the base or various places on the surface of the resting spore	Smooth
de Wildeman 1896	*E. Characearum*	Oögonia of *Chara*	—	—	17–25 μ	1 to 2, at the base of the resting spore	Smooth
Nemec 1912	*E. Brassicae*	*Brassia oleracea*	—	—	3.5–7 μ	1 to 2, usually at the base of the zoösporangium	Smooth
Nemec 1912	*E. Salicorniae*	*Salicornia herbacea*	—	—	4–8.5 μ	1, at the base of the zoösporangium	Double, the inner wall irregular and pointed

rhizoidal system. According to Fisch, regions of the rhizoids may begin to swell and thus develop into secondary, intercalary or terminal zoösporangia or resting spores in the same manner as in the family Cladochytriaceae. Fisch's figures (11, 18, and 19) of this process are not at all convincing, and what he has figured as connected secondary zoösporangia are doubtless nothing but rhizoids of one thallus which pass under or across the sporangia

of another; thus giving the connected appearance. At least his observations have never been confirmed for any other species of *Entophlyctis*. If Fisch's description is correct it is the first case to be recorded, as far as I am aware, in which rhizoids as such have formed secondary growth centers.

Entophlyctis Vaucheriae is the only species to my knowledge, in which germination of the resting spores has been observed. This occurs in the spring of the year according to Fisch. The resting spore absorbs water,



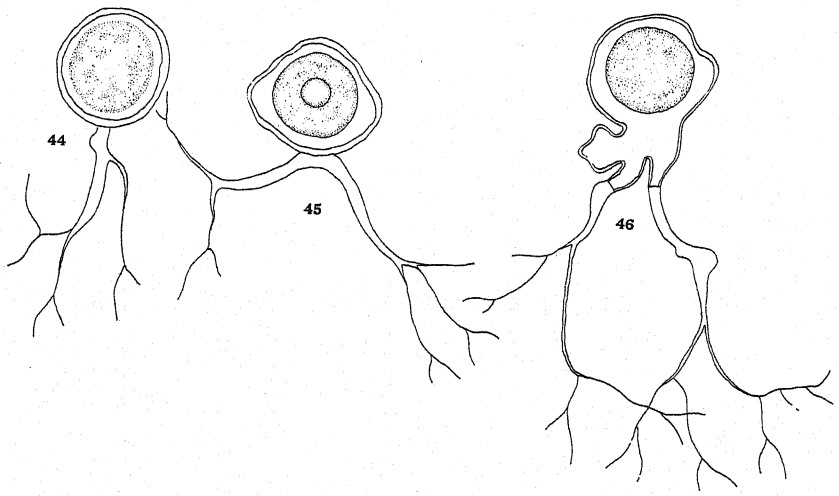
TEXT FIGS. 40–43. *Entophlyctis heliomorpha*. FIG. 40. A mature zoösporangium with zoöspores. FIG. 41. A zoösporangium from a surface view. FIG. 42. A zoöspore. FIG. 43. A resting spore, after Dangeard (1888).

and its granular content becomes homogeneous throughout. The absorption of water causes the outer wall to split and the protoplasm escapes as a round homogeneous vesicle in which streaming is visible. This ceases shortly and bodies or globules (Kerne) appear around which the zoöspores are eventually formed (text fig. 39). Approximately two hours are required after the cessation of streaming for the differentiation and delimitation of the zoöspores.

From Dangeard's and my own descriptions in the literature *E. heliomorpha* appears to be the most ubiquitous of all species. Dangeard described it as *Chytridium helioformis* in 1886 from cells of *Nitella*, *Vaucheria*, and *Chara polyacantha*, but later in 1888 he changed the name to *C. heliomorphum*. I have recorded it from five species of *Chara* and four of *Nitella*, but it is not certain, as noted before, that these are all one species. The fact that Dangeard found it in *Vaucheria* complicates the problem further and raises the question as to whether or not it might be *E. Vaucheriae*. In text figures 40 to 43 are shown his (1888) figures of longitudinal and

surface views of zoösporangia and a resting spore. The species gets its name from the radial arrangement of the rhizoids relative to the fruiting body. Dangeard describes the rhizoids as being six or seven in number, but I have occasionally found zoösporangia and resting spore with only one.

The resting spores of *E. Characearum* were first discovered by Wildeman (1896) in oögonia of *Chara*. According to him the distinguishing characteristics of this species are the limited number, usually one or two, of rhizoids and their position at the base of the spore. Text figures 44 to 46 show some of the resting spores which Wildeman figured. It is evident from these figures that his material was badly shrunken and had perhaps begun to disintegrate. Text figure 46 is particularly significant, because it



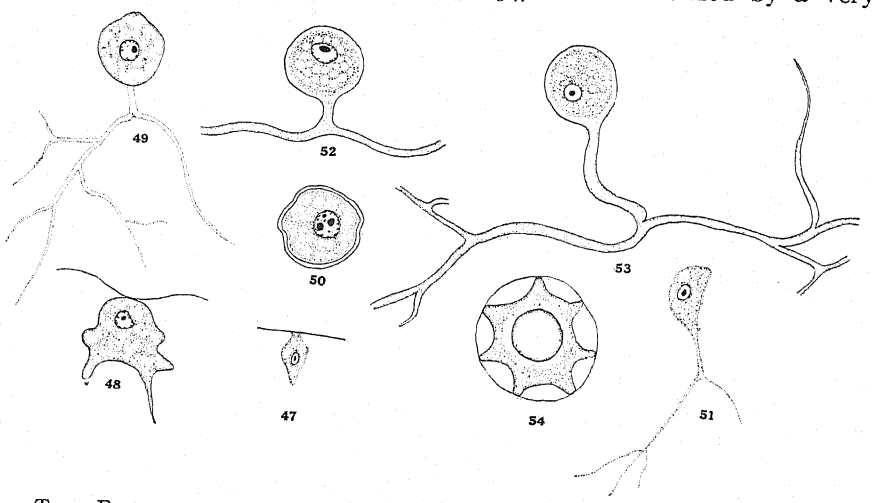
TEXT FIGS. 44–46. Resting spores of *E. Characearum* from oögonia of *Chara*, after de Wildeman (1896).

shows cross walls in the rhizoids separating them from the spore. This is the first record of transverse septa in the rhizoids of the Rhizidiaceae, and it needs further study and confirmation. Wildeman does not figure the zoösporangia and zoöspores, so that it is difficult to diagnose this species further. I have elsewhere called attention to the possibility that it may be *E. heliomorpha*, since this latter species occasionally shows the same characteristics which Wildeman described.

The two remaining species, *E. Brassicae* and *E. Salicorniae*, were discovered and described by Nemec (1912) in roots of *Brassica oleracea* and *Salicornia herbacea*. Their method of development and resting spores are unusual in many respects. According to Nemec, the very young thallus of *E. Brassicae* is at first naked in the host cell like *Olpidium Brassicae* and sends out a number of short blunt and elongated fine pseudopods which are eventually transformed into rhizoids (text figs. 47–49). The thallus shown

in text figure 49 is very similar to those of other species, but the early stage shown in text figure 48 is strikingly unusual. Another conspicuous feature of the zoösporangia, according to Nemec, is that their necks turn away from the cell wall (figs. 9, 13, 14, 17 and 18, Plate XXXVI), and the zoöspores are thus emptied into the host cell. There is nothing unusual in this, it seems to me, since in many species which I have observed the necks may extend in various directions. The resting spores (text fig. 50) are in comparison with those of other species very minute and often no larger than zoöspores, 3.5 to 7 μ. They are uni-nucleate and possess a thick, double contoured smooth wall.

Entophlyctis Salicorniae (text figs. 51–54) is characterized by a very



TEXT FIGS. 47–54. *Entophlyctis Brassicae* and *E. Salicorniae*. FIG. 47. An early infection stage of *E. brassicae*. FIG. 48. A later stage showing the naked thallus and pseudopods. FIG. 49. A monorhizoidal, uni-nucleated thallus. FIG. 50. A resting spore. FIGS. 51, 52, AND 53. Young thalli of *E. Salicorniae*. FIG. 54. A resting spore, after Nemec (1912).

coarse dichotomously branched rhizoidal system and a somewhat star-shaped resting spore. Its method of development and habit of growth is quite like that of other species, but as regards the resting spores they are strikingly unusual both in size and shape. This is the first record of a rough walled resting spore for the genus *Entophlyctis*. In view of this and its similarity in size and shape to the resting spores of *Olpidium Brassicae* and *Endobiella* I am very doubtful as to its identity. It is not improbable that *Salicornia herbacea* is host to these latter parasites, and that Nemec mistook their resting spores to be those of *E. Salicorniae*.

As to the pathogenicity of the various species of *Entophlyctis* they may be either parasitic or saprophytic as far as our present knowledge goes. The majority, however, appear to be saprophytic or at most weakly parasitic.

E. apiculata, nonetheless, according to Braun and Zopf seems to be actively parasitic, since it attacks the swarmspores of *Gloeococcus mucosus* while healthy and in the active swimming stage. Most of the remaining species, *E. Confervae glomeratae*, *E. Cienkowskiana*, *E. bulligera*, *E. heliomorpha* are apparently saprophytes, since they have so far been reported only from dying or dead cells. As to *E. tetrasporum*, *E. Vaucheriae*, *E. Spirogyrae*, *E. Brassicae*, and *E. Salicorniae* very little is known about their pathogenicity.

Summary

1. Attempts to inoculate various species of *Nitella*, *Chara*, *Oedogonium*, *Spirogyra*, *Vaucheria*, *Mougeotia*, and *Hydrodictyon* with *Entophlyctis heliomorpha* from infected Characeae cultures have so far been unsuccessful. Sterile and dead internodes and filaments of these algae were repeatedly placed among the infected cultures, but they remained uninfected.

2. During the progress of these experiments a species of *Entophlyctis* was found parasitizing filaments of *Cladophora* sp. It has many characteristics in common with *E. Cienkowskiana* and *E. Vaucheriae*, but its identity with either of these species is still uncertain.

3. Giant thalli of *Entophlyctis* have been found in *Nitella flexilis*, *Cladophora* sp., and *Elodea* sp. whose rhizoids, zoösporangia, and resting spores are unusually large in size. These may possibly be monstrosities of *E. heliomorpha*, *E. Cienkowskiana*, and *E. Vaucheriae*, or new species.

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DESCRIPTION OF PLATES

All figures were drawn from living material with the aid of a Spencer camera lucida and a Zeiss 2 mm. apochromatic objective N.A. 1.30 and compensating oculars nos. 6 and 8.

PLATE XXXV

FIG. 1. A young short *Cladophora* sp. cell with sixteen thalli of *E. Cienkowskiana* (?). At *A*, *B*, *C*, *D*, and *F* are young thalli to which the spore case and penetration tube are still attached. Six of the chytrid thalli lie within the layers of the host cell wall. The *Cladophora* cell has begun to decay, although a portion of the green plastid is still present with numerous autochthonous starch grains.

FIG. 2. A germinating zoöspore.

FIGS. 3 AND 4. Young thalli with single branched rhizoids.

FIG. 5. A young monorhizoidal incipient zoösporangium with a large central vacuole.

FIG. 6. Another monorhizoidal zoösporangium showing the origin of the sporangial neck.

FIG. 7. A mature zoösporangium which has undergone cleavage into zoöspores. The rhizoids are located at the base.

FIG. 8. Two zoöspores whose cilia are approximately five times their diameter in length.

FIG. 9. A resting spore with three rhizoids localized at the base.

PLATE XXXVI

FIG. 10. A mature polyrhizoidal resting spore from the internode of *N. flexilis*. The rhizoids in this spore arise at several places and are unusually highly branched. The content is finely granular with several globules of variable sizes.

FIG. 11. Another resting spore with two rhizoids at the base.

FIG. 12. Round, oval, and amoeboid shaped zoöspores.

FIG. 13. An early stage in the germination of a zoöspore.

FIG. 14. A small resting spore which is approximately half the size of the one shown in figure 10.

FIG. 15. A large zoösporangium whose neck has branched. The two branches have penetrated the wall and extend more or less parallel along the host cell for a distance of 160 μ.

FIG. 16. A zoösporangium showing the escape of the zoöspores.

PLATE XXXVII

FIG. 17. A mature resting spore and rhizoids from cells of *Elodea* sp. The two rhizoids arise as a pair from the base of the spore and are unusually well branched. The content is coarsely granular, and a large round vacuole or globule occupies the center.

FIG. 18. A monorhizoidal zoösporangium with four necks.

FIG. 19. A small elongated zoösporangium which has undergone cleavage into zoöspores.

FIG. 20. A round zoösporangium showing the escape of the zoöspores.

FIG. 21. An elongated zoösporangium whole wide neck has branched.

FIGS. 22, 23, AND 24. Variations in the size and shape of the resting spores.

FIG. 25. Oval and amoeboid shaped zoöspores.

FIGS. 26 AND 27. Germination and early stages in the formation of the young thallus.

FIG. 28. A young monorhizoidal thallus with the spore case and penetration tube still attached.

PLATE XXXVIII

FIG. 29. A small *Cladophora* cell containing a giant thallus whose rhizoids extend almost the entire length of the cell. At the other end is a thallus of *E. Cienkowskiana.*

FIG. 30. A portion of the main axis of a rhizoid showing the more or less dichotomous habit of branching which gives the axis a somewhat zigzag course.

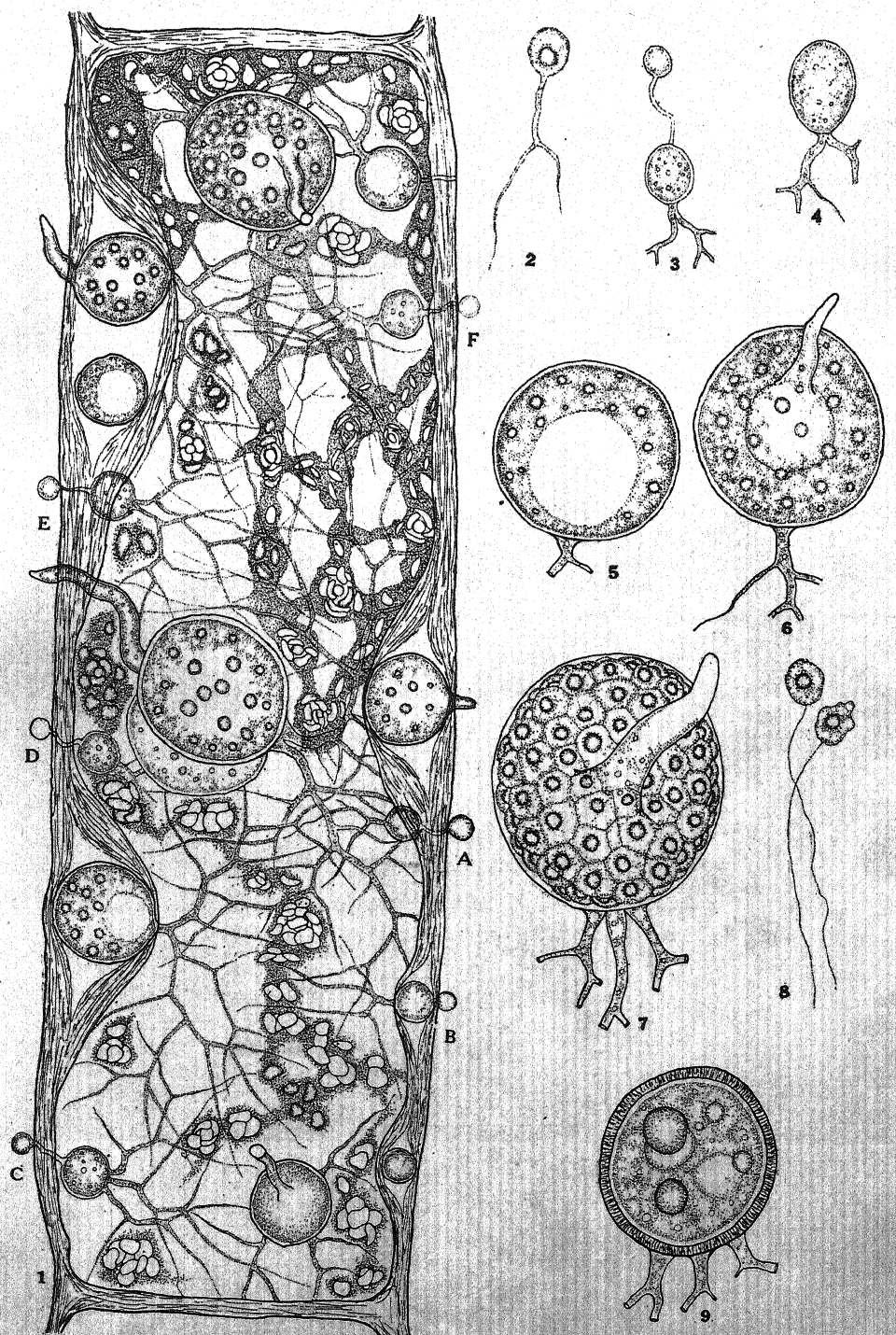
FIG. 31. A large zoösporangium which has undergone cleavage.

FIG. 32. A zoösporangium showing the escape of the zoöspores.

FIG. 33. Zoöspores.

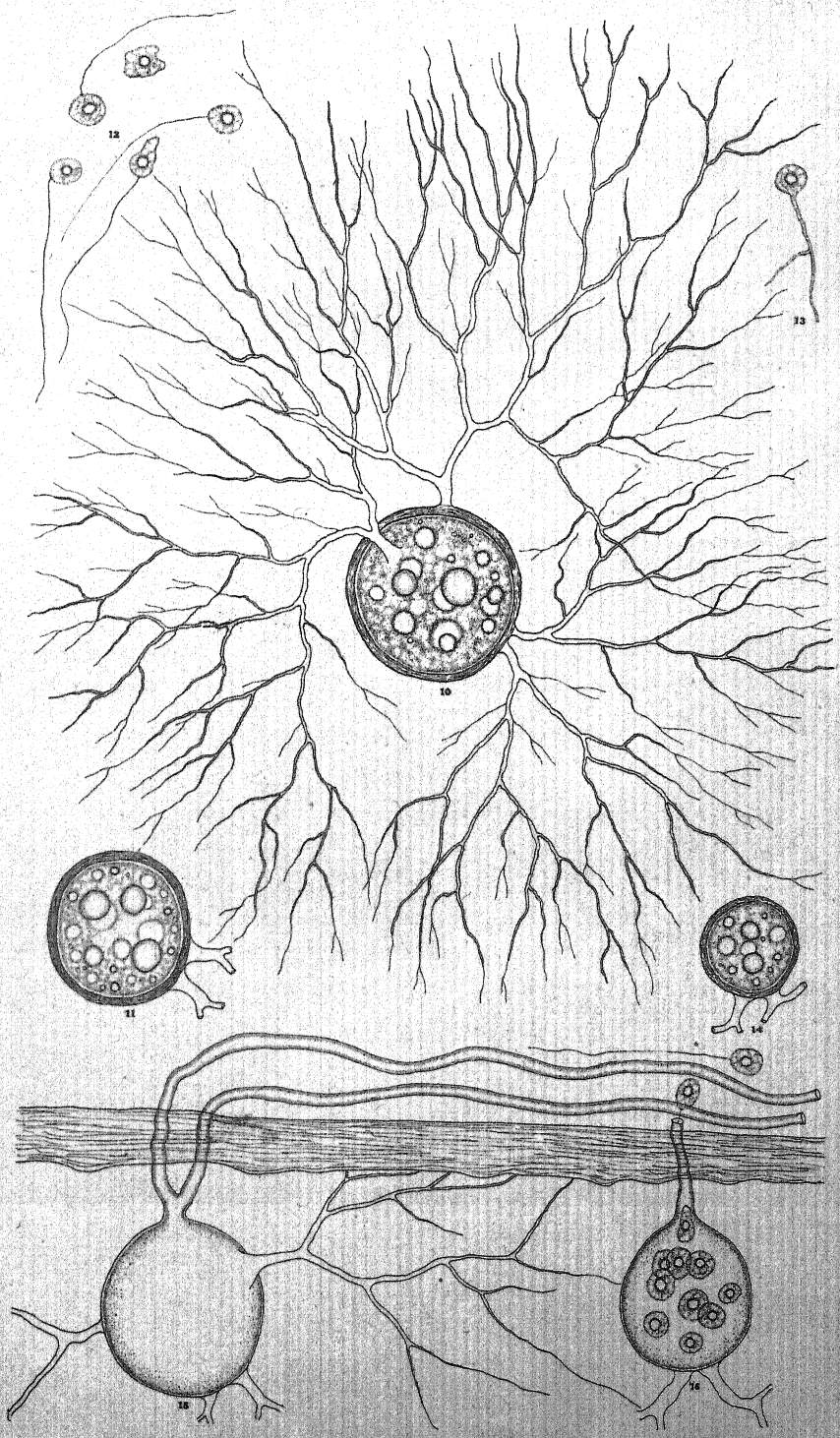
FIG. 34. A germinating zoöspore.

FIGS. 35 AND 36. Young monorhizoidal thalli.

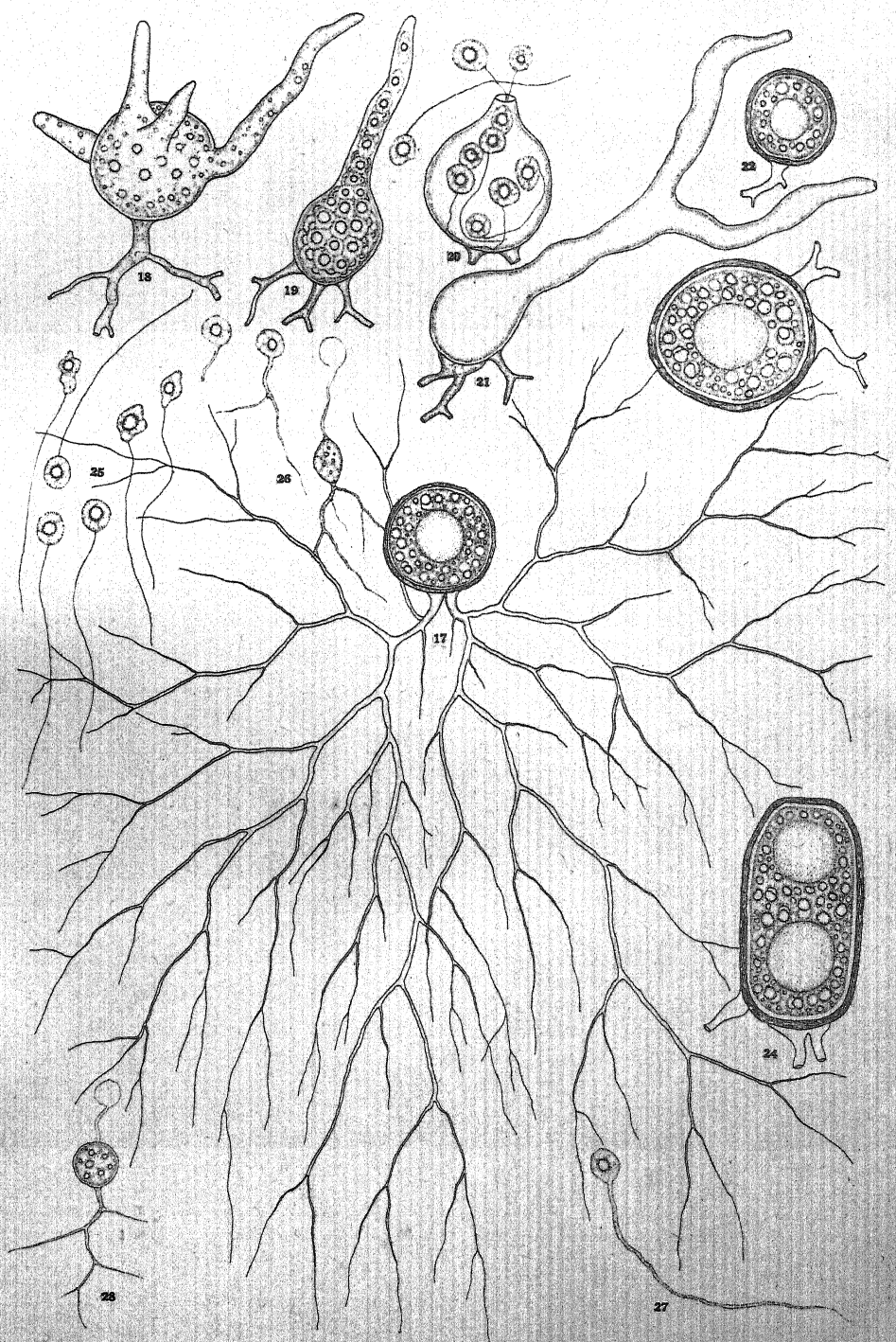


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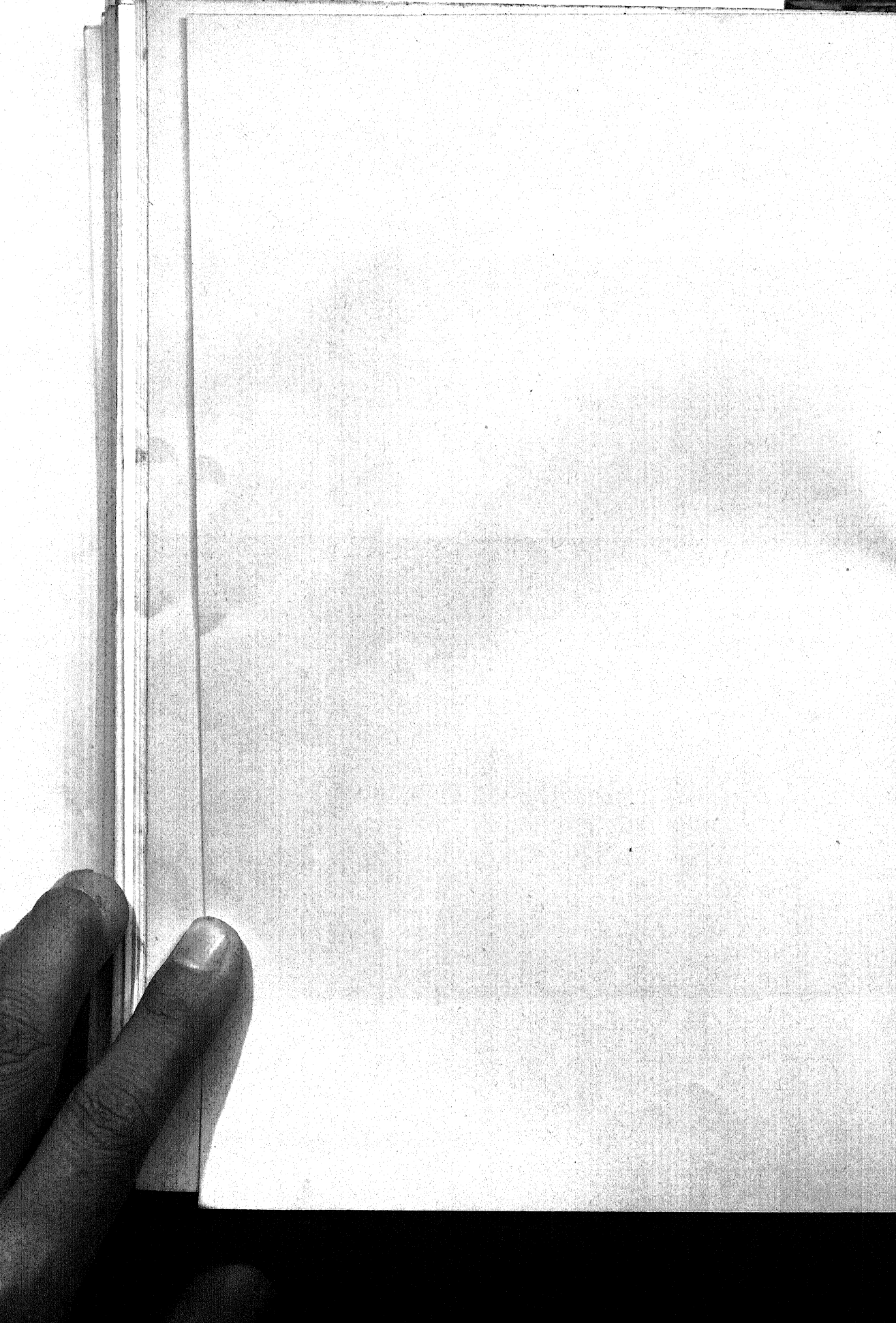


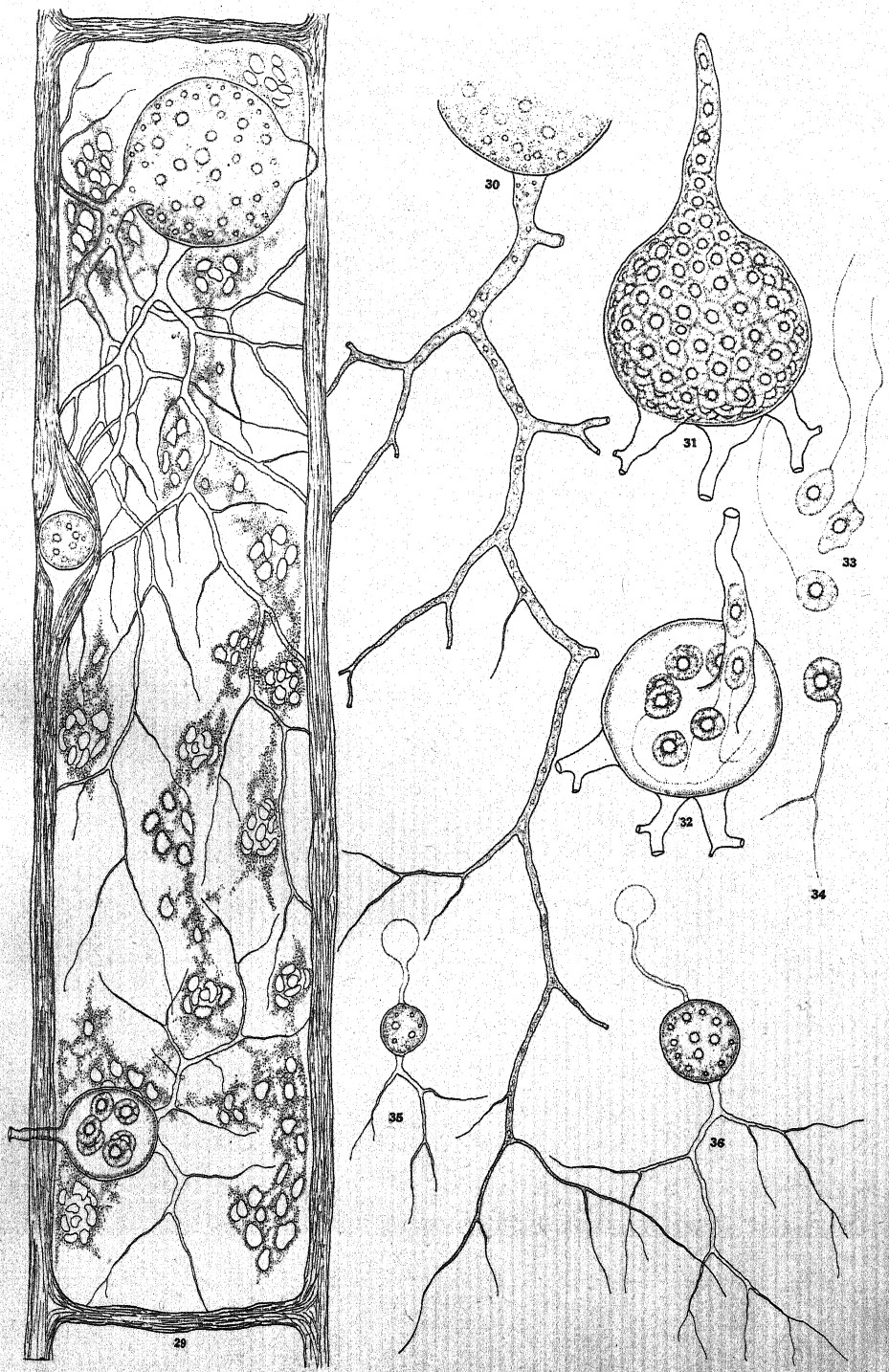


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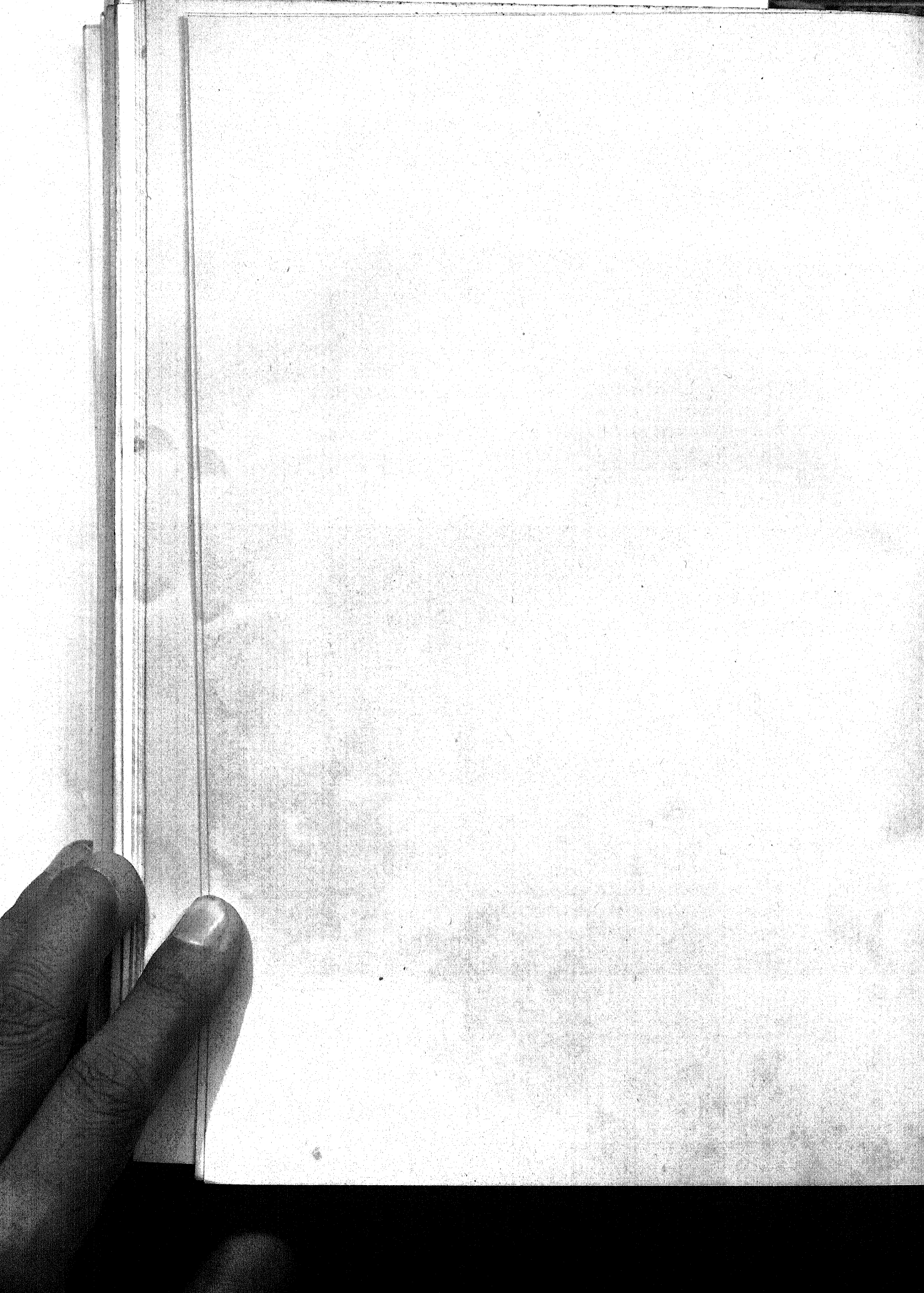


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PATHOLOGICAL, HISTOLOGICAL, AND SYMPTOMATOLOGICAL STUDIES ON PINEAPPLE ROOT ROTS[1]

C. P. SIDERIS AND G. E. PAXTON

(Received for publication August 18, 1930)

INTRODUCTION

So-called "wilt" is a common condition in pineapple fields in Hawaii. The term is not a happy one, since the pineapple plants do not readily droop and wilt. They turn to various shades of yellow, scarlet and brown, sometimes with marked drooping, but more often their loss of character might better be termed shriveling (text fig. 1). In any case, they usually die. If these sick pineapple plants, even in the earliest recognizable stages,



TEXT FIG. 1. Healthy and diseased pineapple plants. *A*, check; *B* and *C*, inoculated with *Nematosporangium rhizophthoron; D* and *E*, inoculated with *Pythium splendens*.

are pulled up, they are found to have but vestiges of a root system. Frequently there are no live roots in the soil and root decay has taken place. It has been frequently assumed that the rotting of the roots was a condition precedent to the "wilting" of the tops, and "wilt" has been looked on as the aërial and visible sign of root destruction. Enough is known of the activities of the nematodes to make it clear that parasitic fungi are by no means the only factors in root destruction. In some fields they may have but little to do with initiating it. Nor would one care to assert that the death of the roots is always the cause of the death of the aërial parts of the

[1] Technical Paper No. 18 of the Experiment Station of the Association of Hawaiian Pineapple Canners, University of Hawaii.

TABLE FOR TEXT FIG. 2

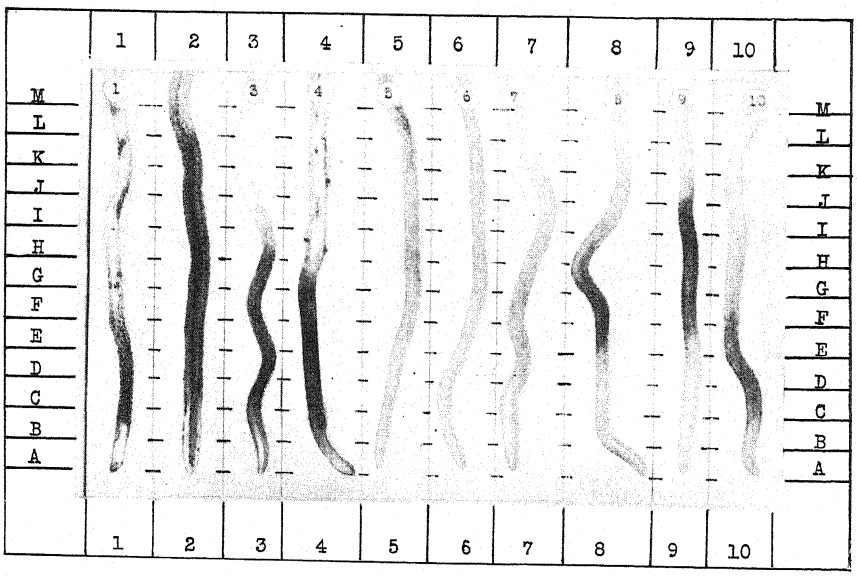
	1 N. rhizophthoron	2 N. polyandron	3 P. diameson	4 P. polycladon	5 P. debaryanum	6 P. debaryanum	7 P. splendens	8 P. artotrogus	9 P. irregularae	10	
M	Vinaceous brown to orange buff	Mars yellow to orange buff		Buff yellow and tawny	Hues of ochraceous buff intermingled with traces of yellowish citrine	Hues of ochraceous buff intermingled with traces of yellowish citrine	Light-ochraceous buff			Light-ochraceous buff	M
L	do	do		do	do	do	do		Ochraceous	do	L
K	do	do		do	do	do	do		buff	do	K
J	do	do		do	do	do	do	Pea green	Deep grey-	do	J
I	do	do	Hues of vinaceous-fawn and olive buff	do	do	do	Hues of light ochraceous buff intermingled with dull opaline green	do	ish blue green	do	I
H	do	do	do		do	do	do	Sage green	do	Ochraceous	H
G	do	do	do	Hues of greyish-olive and clay color intermingled	do	do	do	do	do	buff	G
F	do	do	Intermingled hues of greyish olive and olive buff	do	do	do	do	Artemisia green	Asphodel green	do	F
E	do	do	do	do	do	do	do	do	do	Russian	E
D	do	Light orange yellow	do	do	Massicot-yellow	do	do	Vetiver green	Water green	green	D
C	do	do	do	do	do	do		do	do	do	C
B	do	do	do	do	do	do	Massicot-yellow	Celandine green	do	Water green	B
A	do	do			do		do	do	do	do	A
	1	2	3	4	5	6	7	8	9	10	

plants. Nevertheless, the presence of parasitic fungi is known to be common and widespread, and they are believed to be one of the most important factors in bringing about what is loosely termed "wilt."

This paper lists the organisms which we have demonstrated to occur in rotting roots in the field and to cause root decay when healthy roots are inoculated with their pure cultures. A few closely related organisms, not originally found in field grown plants but capable of parasitizing pineapple roots, are included. It has been found possible to distinguish many of these organisms by their specific behavior in the host tissues, especially in the matter of the formation and characteristics of their oöspores and certain other reproductive organs.

Pineapple Root Rots

The Smooth Cayenne variety of pineapple is the only one grown commercially in Hawaii, and all the plants are members of one clone. They are propagated by setting in the ground either the crowns from the fruits,



Text Fig. 2. Discoloration of pineapple roots by pathogenic species of *Nematosporangium* and *Pythium*. The different pigments were determined by using Ridgway's color standards. For detailed description of colors, see table on opposite page. (Photograph from a painting by Mrs. H. Eller.)

the slips growing out from the fruit stem just below the fruit, or the shoots thrown out by the main stem of the plant. The adventitious roots which grow from the butts of this planting material develop into a root system which has a volume averaging but little more than two percent of that of the aërial parts of the plant. These juvenile roots are supplemented by

new adventitious roots developing in the axils of the leaves. Some of these reach the soil; many do not. The portions of this none too adequate root system which are in the soil are preyed upon by fungi, nematodes, and insects.

In a general way we may group the root rots of the pineapple into:

(1) rapid soft root rot, caused by pythiaceous fungi,
(2) slow hard root rot, caused by Fusaria,
(3) slow soft root rot, caused by Verticillia.

Rapid Soft Root Rot

Root injury caused by pythiaceous organisms is limited in the case of old and highly lignified roots to the succulent tissues near the tip, and in the case of young and succulent roots it usually involves the entire root. The parenchymatous tissues of invaded roots undergo rapid disintegration, whereas the highly lignified tissues escape such a process and form the remains of dead roots. The root symptoms of pythiaceous root rot are the softening and discoloration of the infected tissues. Different species of *Pythium* may produce different hues of green, blue, pink and yellow on the invaded tissues of roots grown in sterilized soil whereas those of *Nematosporangium* produce only a light to dark brown discoloration (text fig. 2). Such discolorations, however, are difficult to detect in soils contaminated with other organisms besides the primary pathogenes.

Slow Hard Root Rot

The symptoms of this root rot are characterized by a hardening and discoloration of the infected tissues, which may change to a dark brown or grayish brown color (text figs. 23 and 24). Pigmentation starts at the point of the initial infection and spreads slowly towards the pericycle of the root. The cortical tissues of the root show the first symptoms of infection at the point of emergence of the young laterals. Infection may start either on the emerging tips of the young laterals or on the few dead cells of the cortex which result during the process of emergence. In the majority of cases, however, initial infection starts through the dead tissues of the cortex.

Slow Soft Root Rot

This rot cannot be differentiated very easily from the one caused by pythiaceous organisms except for its rate of development, which is considerably lower in this case. Infection may start at the tips of either the main roots or those of the laterals. Infected roots become opaque and soft with a yellowish brown discoloration.

The death of entire roots may be brought about either by the activities of the primary invaders or by those of secondary invaders. Some of the roots that escape death produce laterals which are able to take care of the

mineral and water requirements of the plants. It is believed that secondary invaders may constitute a factor in the death of roots invaded primarily by pythiaceous pathogenes. These secondary organisms, mostly species of *Fusarium* and related genera, first establish themselves on the dead tissues left behind by the original invader, and may injure the adjacent healthy tissues either through the production of toxic products or through the invasion of their vascular bundles.

The manifestations of root failure on the aërial parts of plants become apparent by the discoloration, drooping, and withering of the leaves. Leaf discoloration is more pronounced on those exposed to sunlight than on those in the shade. There is no sharp line of demarkation between the symptoms on the leaves due to root rot and those due to root failure by other causes.

History and Distribution of the Disease

If we consider root rot synonymous with pineapple wilt, except in those cases where nematodes, insects, or soil conditions are wholly responsible for its development, we can say that it occurs in most of the pineapple growing countries (10, 15, 28, 29, 32, 33, and 34). It is more common on the cultivated varieties than on the wild ones.

Carpenter (2) was the first in Hawaii to attribute pineapple root rot to pythiaceous organisms and also to reproduce the disease and point out its relationship to "pineapple wilt" and the "Lahaina disease" of sugar cane. In 1925 the senior author (18) obtained pythiaceous organisms from the diseased roots of pineapple plants, besides members of the genera *Fusarium* and *Verticillium*.

The possible association of *Fusarium* with pineapple wilt has been considered also by previous investigators. Larsen (8) comments as follows: "It is generally held that pineapple wilt is a similar disease to cotton wilt, and is caused by a fungus organism that enters the roots and grows into the main stalk of the plant, travelling through the vascular bundles. This assumption is based on the fact that a species of *Fusarium* is often found in the roots of wilted pineapples, while a *Fusarium* is responsible for wilt disease in certain other plants. This evidence, however, becomes rather weak when we consider that various types of *Fusarium* are among the most common soil-inhabiting fungi and can at any time be found in diseased roots from healthy plants as well as from plants affected with wilt." Weber (34) also says: "There is little doubt that wilt is caused by a parasitic fungus attacking the roots in a manner similar to *Fusarium* disease affecting cotton, melon, squash, etc." The author (17) has observed that a species of *Fusarium* of the section Elegans was able to cause injury to pineapple roots only when the plants were grown in water cultures of pH values ranging above 6.5 but not below; in this particular case, the relative acidity of the culture playing a more important rôle by weakening the tissues than the parasite.

Pathological and Histological Technique

The technique employed for the isolation of pythiaceous organisms from diseased root tissues has undergone many modifications and improvements since the beginning of these studies.

The senior author (17) in his early isolations of pathogenes from diseased roots employed the tissues of the cortex and the stele that were adjacent to the healthy tissues and discarded all the thoroughly decomposed parts. Such tissues were planted in nutrient dextrose agar media. The results were a very high percentage of *Fusarium*, *Trichoderma*, and *Penicillium* and a low one of *Pythium*, *Verticillium* and a few other organisms.

This technique was later modified. The stele, especially that portion adjacent to the healthy tissues, was dissected out as carefully and as aseptically as possible and plated in the culture media above mentioned. The cortex was discarded merely to avoid contaminating organisms. The results obtained by this method indicated a greater majority of species of *Fusarium* than previously obtained.

In 1927 C. W. Carpenter described to the junior author his method of obtaining pythiaceous organisms from sugar cane roots, which consisted in plating out on corn meal agar the diseased and almost decomposed tissues of the cortex of the root tip. This method was tried out, and led to more frequent isolations of pythiaceous organisms. Carpenter's method was finally modified and considerably improved by the introduction of the root study box (4, 22). Soil from diseased areas was placed in the Comstock type root study boxes and planted to pineapples. The soil was frequently watered to encourage the rapid development of roots and also that of the fungi, especially pythiaceous organisms of the soil. The roots visible through the glass of the sides were examined daily, frequently with the aid of a Greenough type binocular microscope mounted on a dermatoscope stand. The first symptoms of disease could be easily detected by the discoloration of the tissues. By lifting the glass of the box, roots of different stages of infection were obtained from the soil and either plated out on *Carica papaya* agar media or used for microscopic study. Early infections, *i.e.* one to three days old, yielded pure cultures of pythiaceous organisms in some of the cases where typical pythiaceous root rot was seen. Infections, three to ten days old, yielded species of either *Fusarium* or *Rhizoctonia*, *Trichoderma*, *Penicillium*, *Alternaria*, *Phoma*, etc., in addition to the primary pythiaceous invader or invaders.

All pythiaceous organisms obtained in pure cultures by this method were used for the inoculation of pineapple roots grown in steam sterilized soil, and reisolations made from the diseased roots arising from such inoculations.

The histological preparations were made from roots grown in sterilized soil and inoculated with pure cultures of the different organisms. Most of

these preparations from which the various photographs were taken were made by Dr. R. C. Whitman of the University of Colorado. A few such photographs were taken of free-hand and unstained sections prepared by the senior author.

For the sake of completeness a brief outline of Dr. Whitman's histological technique is inserted.

The diseased roots were first carefully washed by using a fine but powerful spray and a small camel's hair brush to remove the adhering soil particles. The tissues were given the following treatments:

1. Fixation, in Zenker's fluid for 24 hours.
2. Washing in running tap water for 24 hours.
3. Dehydration, 24 hours in each of the following: alcohol 10%, 20%, 30%, 40%, 50% alcohol plus anilin oil, 70% alcohol plus anilin oil, anilin oil.
4. Clearing in xylol.
5. Embedding in paraffin.
6. Cutting sections 10 μ thick.
7. Removing paraffin and preparing the sections for staining in the following steps, each about one minute:
   a, xylol; *b*, xylol; *c*, absolute alcohol; *d*, 95% alcohol; *e*, 40% to 60% alcohol; *f*, water.
8. Staining in Mallory's phosphotungstic acid hematoxylin. Previous to staining the sections are treated as follows:
   a, $\frac{1}{4}$% potassium permanganate in water (freshly prepared) 5 minutes; *b*, rinse in water; *c*, 5% oxalic acid 5 minutes; *d*, washing thoroughly in several changes in water to remove oxalic acid; *e*, Mallory's phosphotungstic acid hematoxylin stain, 24 hours or longer; *f*, transfer from stain to 95% alcohol, 1–2 minutes; *g*, in absolute alcohol, two changes, $\frac{1}{2}$ minute each; *h*, oil cedar, 2 or 3 minutes; *i*, xylol two changes.
9. Mounting in balsam.

Other stains such as methylene blue, Fleming's triple, and Gram were used, but not with as satisfactory results as Mallory's phosphotungstic acid hematoxylin.

A number of advantages can be claimed for the stained preparations. They enabled the authors to ascertain the initial path of invasion and the extent of such invasion into the tissues of the host by the various pathogenes.

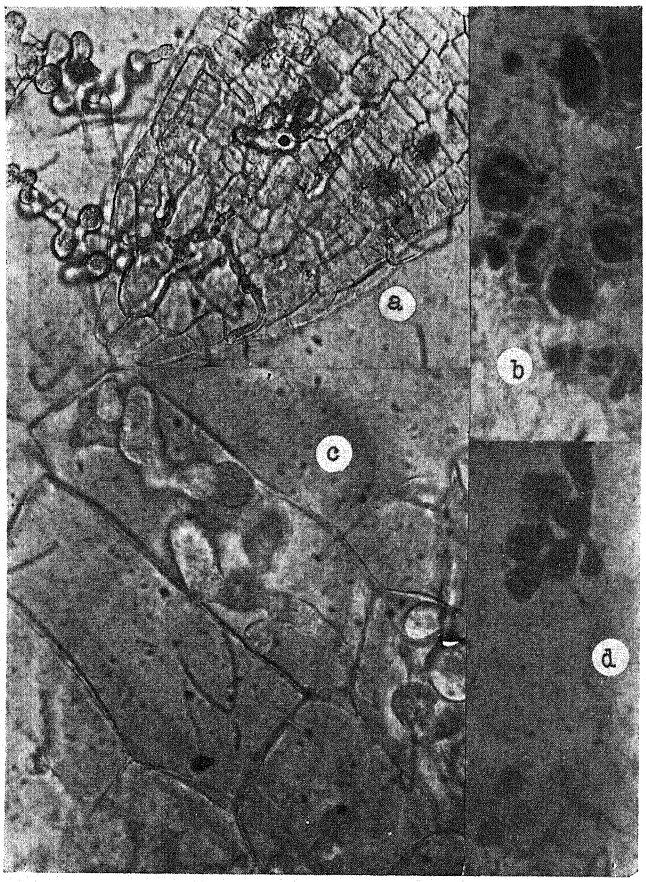
Behavior of Pythiaceous Fungi in Pineapple Root Rot

Four genera of Phycomycetes have been demonstrated to cause root rot of pineapples—*Nematosporangium*, *Pythium*, *Pseudopythium*, and *Phytophthora*.

The various species of *Nematosporangium* are, as a group, more pathogenic on pineapple roots than those of *Pythium*. They promptly invade the roots as they come in contact with them and break down the succulent tissues, producing a soft rot and brown discoloration. The pathogene invades the tissues of the root usually through the tip at a point of 1 to 5 mm. above the very apex. Symptoms of infection have often been detected within 24 hours after inoculation. Lignified tissues are very seldom invaded. The members of *Nematosporangium* are readily differ-

entiated in the tissues of hosts by their plasmatoögoses inside the cells of the host.

The various members of *Pythium* are, as a group, mild parasites of pineapple roots, except for *P. diameson* and *P. splendens* which are as aggressive as some of the Nematosporangia. Their invasion of pineapple roots is characteristically limited to the succulent tissues of the root, but they may also be found, although rarely in the fibrovascular bundles of the roots. They are differentiated in the tissues of hosts from *Nematosporangium* by the absence of plasmatoögoses, and from *Pseudopythium* and

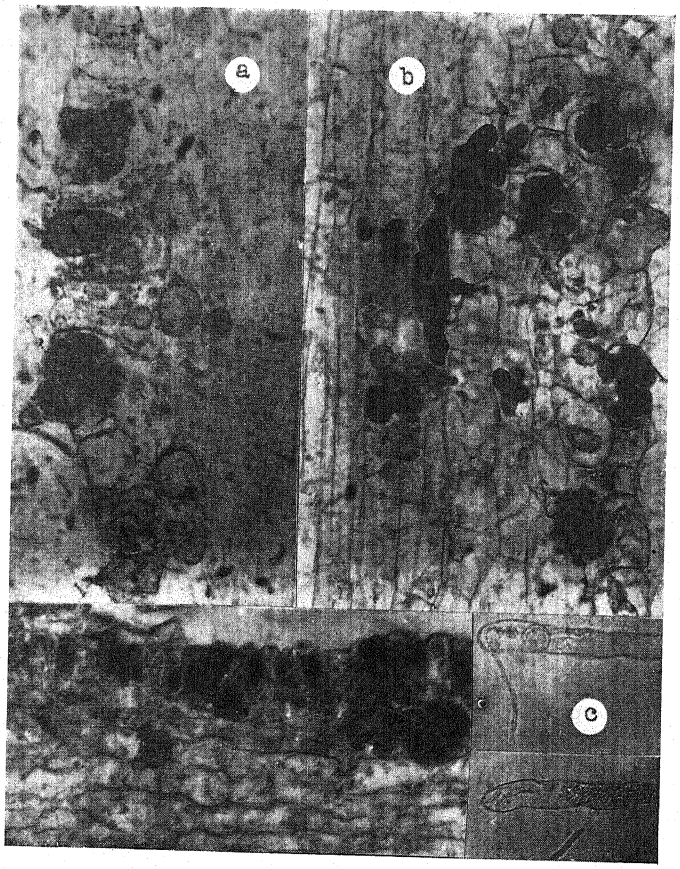


TEXT FIG. 3. *Nematosporangium polyandron*. *a*, root tip invaded by the parasite (× 300); *b*, plasmatoögoses in root tissues (× 300); *c*, *d*, plasmatoögoses (× 600).

Phytophthora by the presence of oöspores and morphological differences of their hyphae. Different species of *Pythium* cause tissue discolorations of various hues of blue, green, pink and brown, if the pineapple roots are

grown in sterile soil and protected from other biological contaminations. Different species produce different hues.

Pseudopythium, represented in these studies only by one species, may be differentiated intracellularly from *Phytophthora Meadii* and *Ph. melongenae* by the coarse, thick, hyphae showing little or no ramification. The hyphae

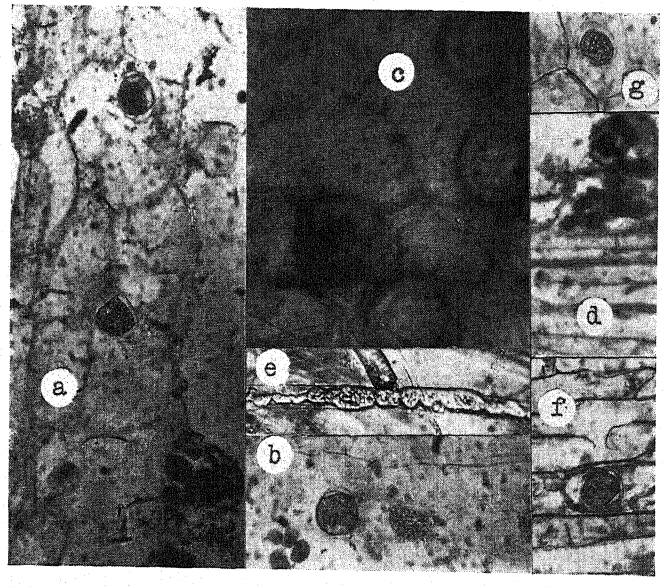


TEXT FIG. 4. *Nematosporangium thysanohyphalon*. *a*, plasmatoögoses and abortive oögonia in root tissues (× 300); *b*, plasmatoögoses (× 300); *c*, germination of a zoöspore through a root hair (× 600).

of both species of *Phytophthora* are of relatively smaller diameter than those of *Pseudopythium* and ramify extensively producing either small branches or appressoria-like bodies.

Interspecific Differentiation of Nematosporangium in Pineapple Root Tissues

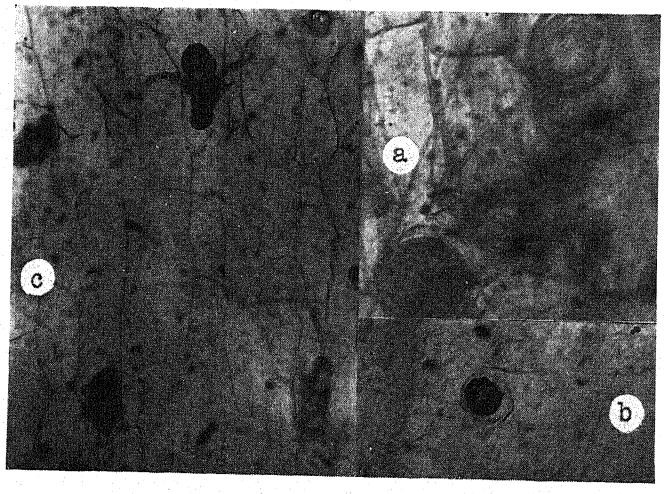
A brief mention of interspecific differentiation of *Nematosporangium* in the tissues of hosts has been presented in a previous paper by the senior



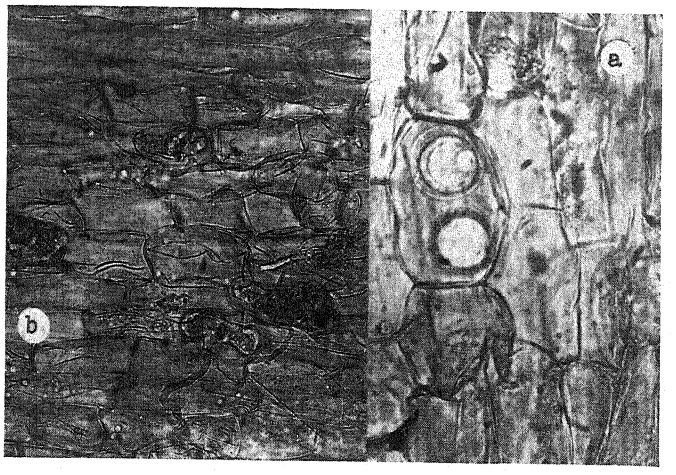
TEXT FIG. 5. *Nematosporangium rhizophthoron.* *a*, *b*, *d*, *f*, and *g*, plasmatoögoses and oöspores in root tissues (× 300); *c*, oöspores in root tissues (× 600); *e*, root hair completely filled by plasmatoögoses.

author (25). The morphology of the oöspores and plasmatoögoses formed, in the studies referred to, the basis of such a differentiation (text figs. 3–8).

All the species of *Nematosporangium* employed in these studies were found to be intracellular parasites. The rate of invasion and killing of



TEXT FIG. 6. *Nematosporangium hyphalosticton.* *a*, oöspores in root tissues (× 600); *b* and *c* oöspores and plasmatoögoses (× 300).



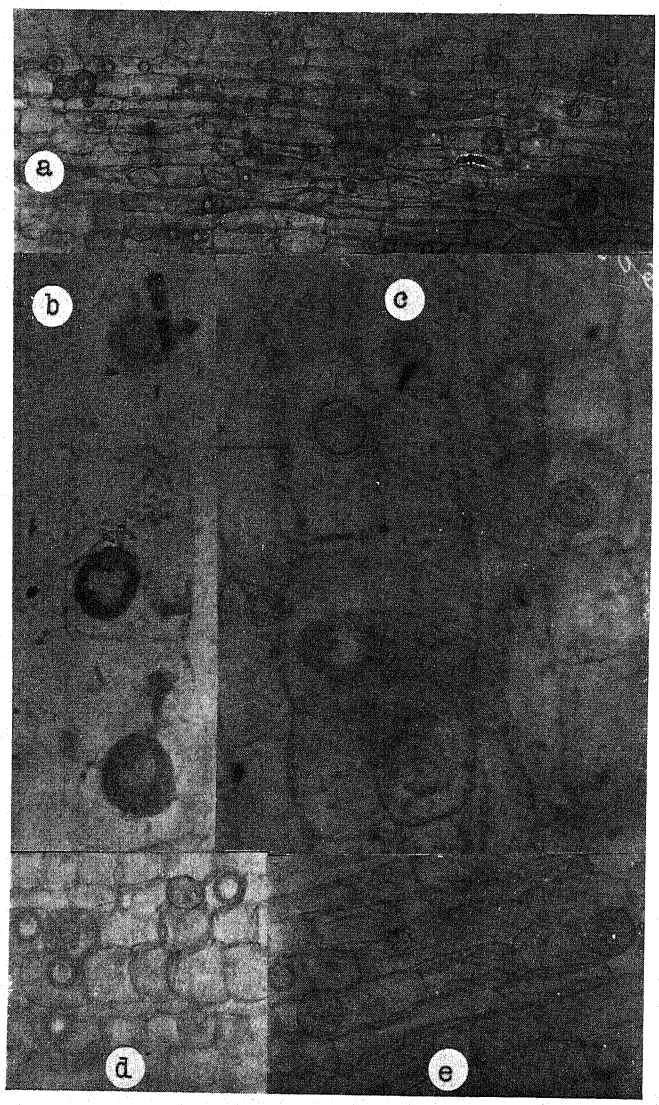
TEXT FIG. 7. *Nematosporangium aphanidermatum*. *a*, oöspores in root tissues (× 600); *b*, plasmatoögoses in root tissues (× 300).

the tissues of the pineapple roots is very rapid. The parasites soon after they have invaded the tissues produce plasmatoögoses, which may fill either in part or completely the invaded cells of the root. These organs germinate soon after their full development or later, and may give rise to either zoösporangia or vegetative hyphae which propagate the organism very rapidly. The development of sexual organs, namely, oögonia and antheridia or oöspores, is more regular with certain groups than with others.



TEXT FIG. 8. *Nematosporangium indigoferae*. Oöspores in root tissues (× 600).

For instance, the species of the subsection Bradyspora produce few of these organs very rarely and rather irregularly. The members of the subsection Hemibradyspora produce more of them but irregularly, while those of the

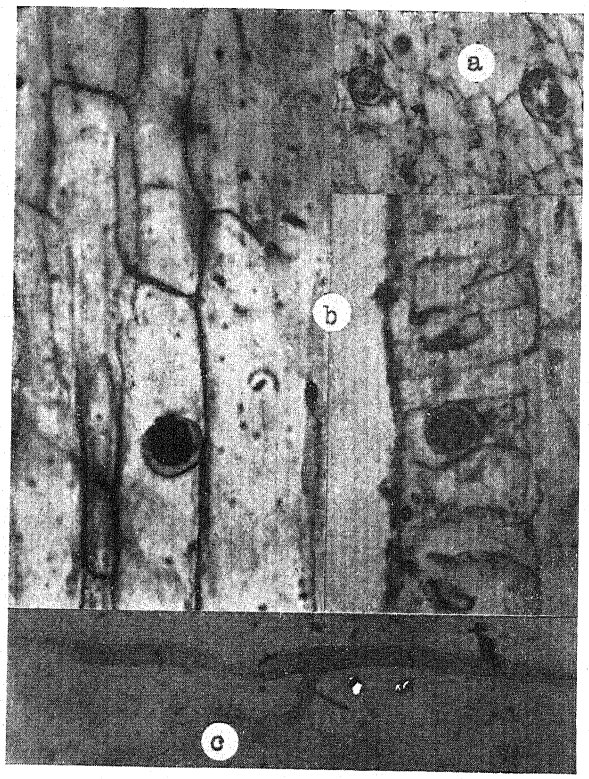


TEXT FIG. 9. *Pythium diameson*. *a*, intracellular prosporangia and oöspores (× 150); *b* and *e*, intracellular prosporangia (× 600 and × 300); *c*, oöspores (× 600); *d*, oöspores and prosporangia (× 300).

subsection Tachyspora produce many of them regularly. The development of sexual organs, their numbers, and also the relative size of the plas-

matoögoses are influenced by the available food substances of the invaded root tissues of the host.

Therefore, a differentiation of the various species of *Nematosporangium* in the diseased tissues of pineapple roots is possible if such species belong at least to different subsections. For instance, differentiation between

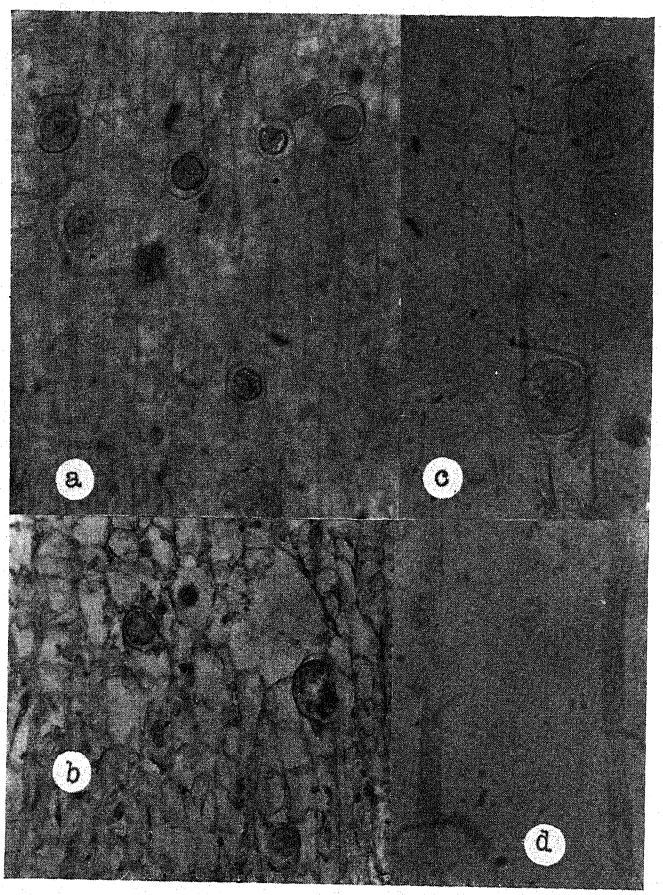


TEXT FIG. 10. *Pythium splendens*. *b* and *c*, oöspores and hyphae in root tissues (× 600); *a*, oöspores (× 300).

N. arrhenomanes and *N. spaniogamon* is impossible, because both of these organisms belong in the same subsection. It is possible to differentiate, however, these organisms from *N. hyphalosticton*, *N. polyandron*, or *N. thysanohyphalon* (text figs. 3 and 4) on the basis of the frequency of development of their sexual organs, the latter producing such organs more often than the former. Differentiation of the members of the subsection Tachyspora (text figs. 5 and 6) from all the above species is easy, because they develop sexual organs in the tissues of hosts very regularly and in great numbers. *N. aphanidermatum* (text fig. 7) and *N. indigoferae* (text fig. 8) may be differentiated from all the above mentioned species by the smaller

size of their oöspores. Differentiation between *N. aphanidermatum* and *N. indigoferae* is possible through the development of plasmatoögoses. These organs are produced very rarely by *N. indigoferae* but often and in rather large sizes by *N. aphanidermatum*.

In table 1 are summarized the behavior of eight species of *Nematosporangium* found in pineapple roots. In addition three species which were not originally isolated from diseased pineapple roots are included since they were found capable of invading them when given the opportunity.



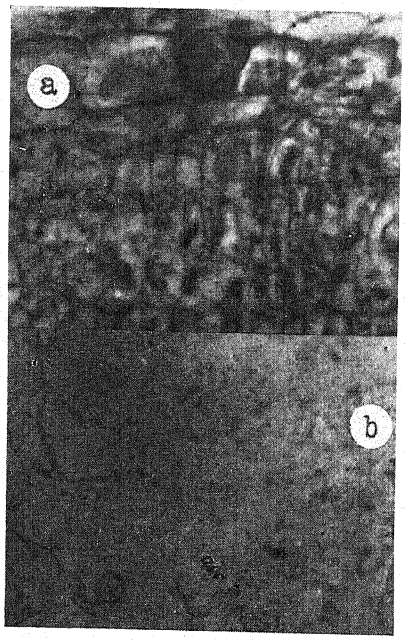
TEXT FIG. 11. *Pythium debaryanum*. *a* and *b*, oöspores (× 300); *c*, oöspores (× 600); *d*, hyphae (× 600).

Interspecific Differentiation of Pythium in Pineapple Tissues

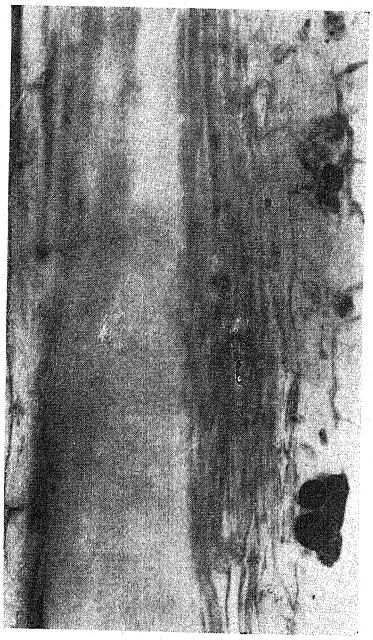
The symptoms of infected pineapple roots by species of *Pythium* consist mainly of soft rot and discoloration. The rate of development of the soft rot varies with different species and also with different roots. With some

TABLE 1. *Characteristic Behavior of Eleven Species of Nematosporangium*

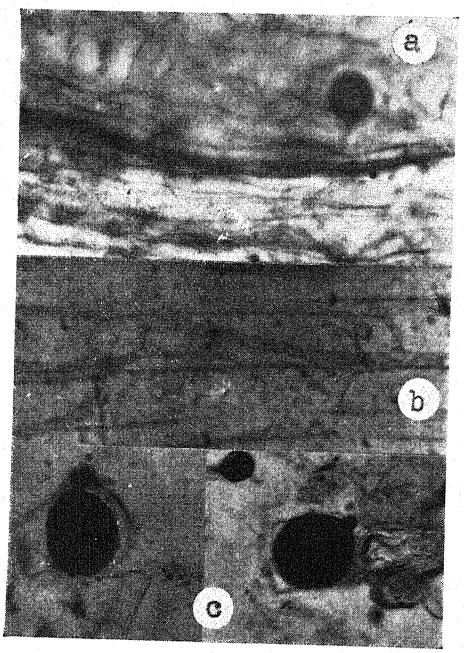
Nematosporangium Species	Localities Found	Parasitism	Oöspores in Tissues	Plasmatoögoses	Remarks
N. arrhenomanes var. *hawaiiensis*	Oahu, 4 fields	Aggressive on root tips intravascular	Rarely	Many and large	Moderately distributed
N. spaniogamon	Oahu, 1 field	Aggressive	Never observed	Many and large	Rare in the fields
N. hyphalosticton	Oahu, Maui Molokai, Lanai	Very aggressive, killing succulent roots	Present	Many and large	Arid lands usually, widely distributed
N. polyandron	Oahu, Maui, Molokai	Very aggressive	Present, less than last above	Many and large	Widely distributed
N. thysanohyphalon	Molokai	Aggressive	Same	Present	Rare
N. rhizophthoron	Oahu, Maui, Molokai, Kauai, Lanai	Very aggressive in succulent tissues	Great number largely in epidermis	Present	Distinguished by the large number of perfect oöspores. Most important species
N. leucosticton	Oahu, not in pineapple fields	Will invade pineapples	Present, some abortive	Present	Isolated from a species of *Bilbergia* at the greenhouse
N. leiopyphon	Oahu, 2 fields	Moderately aggressive	Present, many abortive	Few and small	Majority of oöspores in tissues are abortive
N. epiphanosporon	Oahu, 1 field	Moderately aggressive	Present, but mostly abortive	Few and small	Oöspores largely abortive, also oögonia
N. aphanidermatum	Oahu, not in pineapple fields	Aggressive on inoculation	Present	Present	Oöspores smallest of any species noted. Culture obtained from the roots of *Carica papaya*
N. indigoferae	Culture sent by MacRae from India. Not in pineapple fields	Weak	Present	None	From the roots of *Ananus sativus*. Weak parasite



TEXT FIG. 12. *Pythium euthyhyphon.* *a* and *b*, oöspores (× 600 and × 300).



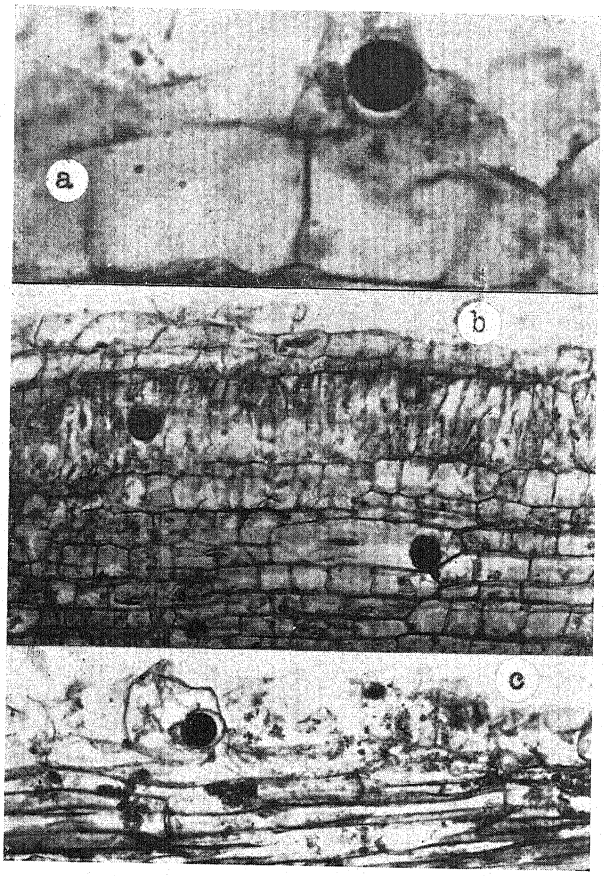
TEXT FIG. 13. *Pythium teratosporon.* Irregular bodies (× 300).



TEXT FIG. 14. *Pythium polycladon.* *a*, oöspores; *b*, hyphae, and *c*, prosporangia (?) (× 600).

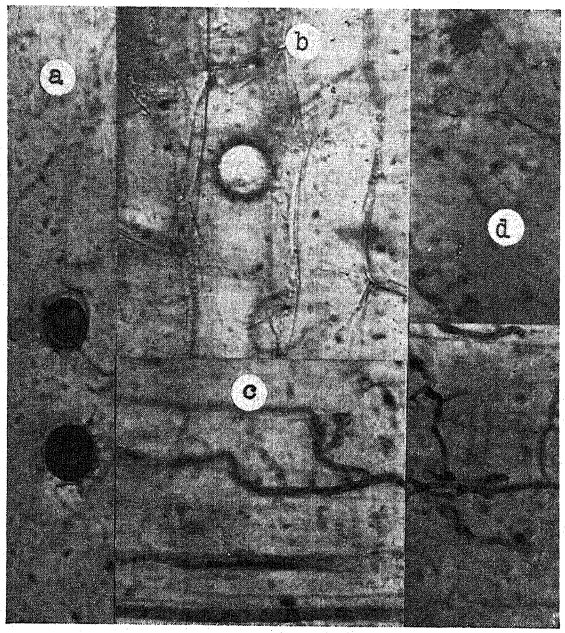
species it is very high and with others very low and almost insignificant. For instance, *P. diameson* and *P. splendens* are highly parasitic in comparison to such species as *P. acanthophoron*, which can be easily classed either as a facultative parasite or a saprophyte.

The different species of *Pythium* involved in pineapple root rot may be differentiated in the diseased tissues by the absence or presence of oöspores

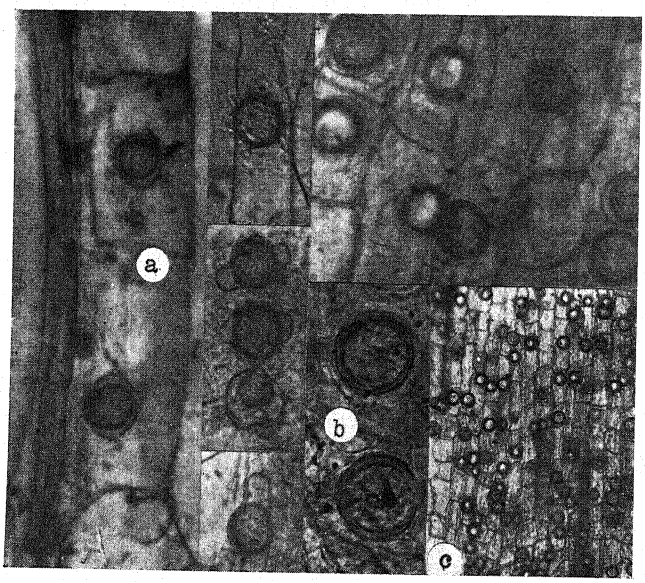


TEXT FIG. 15. *Pythium ascophallon*. *a*, oöspores (× 600); *b* and *c*, oöspores (× 300).

and by their morphology (26) (text figs. 9–20). Oöspores may be differentiated in the tissues of the host: (1) by their size, large or small; (2) by their position in relation to the oögonium, plerotic or aplerotic; and (3) by the character of their surfaces, smooth, spiny, or polymorphic. The development of intracellular prosporangia, as in *P. diameson*, is a very reliable character, but does not occur in many species. The production of different hues on diseased roots by different species cannot be relied on for the differ-

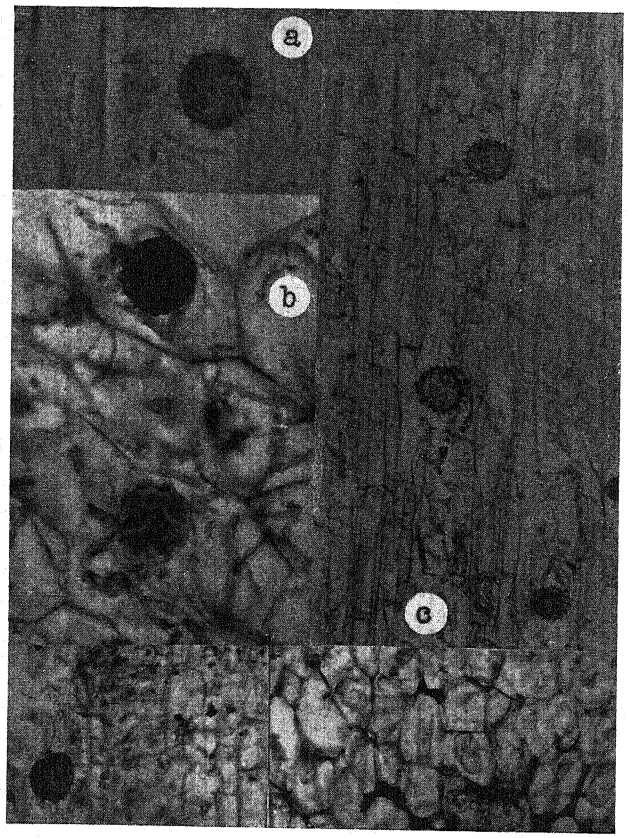


TEXT FIG. 16. *Pythium artotrogus*. *a*, *b*, and *c*, intracellular oöspores and hyphae (× 600); *d*, intracellular oöspores (× 300).



TEXT FIG. 17. *Pythium irregulare*. Intracellular oöspores, *a* (× 600), *b* (× 800) and *c* (× 150).

entiation of such species, because it is susceptible to changes by contaminating organisms and also by different hydrogen-ion concentrations. The type of the antheridium cannot be employed for the differentiation of different species, because a morphological differentiation of this organ by the microscope in the tissues of the host is extremely difficult. The species with smooth oöspores can be differentiated very readily from those with spiny oöspores, and likewise, those with aplerotic from those with plerotic



TEXT FIG. 18. *Pythium acanthophoron*. *a* and *b*, oöspores in root tissues (× 600); *c*, oöspores (× 300).

oöspores. For instance, the spiny species *P. acanthophoron* (text fig. 18), *P. irregularae* (fig. 17), *P. mamillatum*, and *P. artotrogus* (fig. 16) can be differentiated from the smooth species *P. ascophallon* (fig. 15), *P. polycladon* (fig. 14), *P. euthyhyphon* (fig. 12), *P. debaryanum* (fig. 11), and *P. splendens* (fig. 10). Species with plerotic oöspores, such as *P. diameson* (fig. 9), can be differentiated readily from all of the species mentioned above with smooth but aplerotic oöspores. Differentiation between the members of the group

with spiny oöspores can be made on the basis of the size and number of spines of their oöspores. For instance, *P. irregularae* may be differentiated from *P. artotrogus* by the irregular number of spines on its oöspores. This number may vary between none and many, while on the oöspores of *P. artotrogus* the number of spines is always high and more or less constant. *P. acanthophoron* may be differentiated from both these species by its relatively shorter spines. Differentiation between the members of the



TEXT FIG. 19. *Pythium intermedium.* Hyphae in root tissues (× 600).



TEXT FIG. 20. *Pythium mamillatum.* Hyphae in root tissues (× 600).

group with smooth and aplerotic oöspores is not very easy, because of the lack of highly contrasting features, except relative size, between the oöspores.

The various illustrations (text figs. 9–20) show some of these features very clearly.

The characteristic behaviors of twelve species of *Pythium* in their invasion of pineapple roots are summarized in table 2. Eight of these were isolated from field grown roots, one appeared on pineapple plants growing in sand culture in the greenhouse, three were from other sources.

Pseudopythium and Phytophthora in the Tissues of Pineapple Roots

Neither *Pseudopythium* nor the *Phytophthora* species concerned in these studies have ever been observed to produce either sexual or asexual reproductive organs in the tissues of the roots of pineapple plants. Therefore, a differentiation of these organisms on the basis of the morphology of their reproductive organs as in *Nematosporangium* and *Pythium* is impossible.

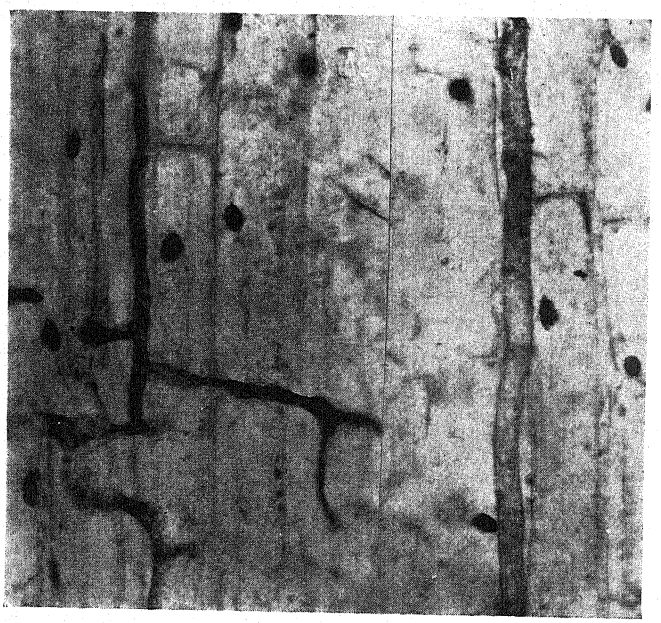
TABLE 2. *Characteristic Behavior of Twelve Species of Pythium Parasitic on Pineapple Roots*

Pythium Species	Localities Found	Parasitism	Oöspores in Tissues	Remarks
P. ascophallon	Oahu, 2 fields	Weak, intracellular	Few, aplerotic	Little economic importance
P. polycladon	Oahu, University Farm. Not in pineapple fields	Very weak	Rare, smooth, aplerotic, occasional prosporangia	Isolated from castor bean roots
P. euthyhyphon	Oahu	Weak intracellular and intravascular	Rare	Not important
P. intermedium	Oahu, greenhouse	Weak intracellular and intravascular	Not observed	Not important
P. acanthophoron	Oahu	Very weak	Many, spiny	Rare
P. debaryanum	Oahu and Maui	Moderate, intracellular and vascular	Many, smooth, aplerotic, no prosporangia	Of some importance
P. splendens	Oahu, many fields	Very aggressive often killing entire root	Rare, smooth, aplerotic, few prosporangia	Abundant and important
P. teratosporon	Oahu, not in pineapple fields	Weak	Rare	Isolated from spinach. Not important
P. irregulare	Oahu, 6 fields	Aggressive	Many polymorphic	Of some importance
P. mamillatum	Holland—not in pineapple fields	Weak	Not observed	Culture from Centraal Bureau voor Schimmelcultures. Not important
P. artotrogus	Oahu and Maui, in pineapple fields	Aggressive, intracellular and vascular	Present, spiny spines longer than acanthophoron	Of some importance
P. diameson	Maui, in pineapple fields	Most aggressive Pythium	Many, small, plerotic, many prosporangia in exodermis	Very important when it occurs

Pseudopythium (27) can be readily differentiated from *Phytophthora* by the relatively great diameter of its hyphae, but the two different species of *Phytophthora* employed in these studies cannot be differentiated by any reliable morphological character.

Ps. phytophthoron Sideris

This organism was found only in one field, in the Waialua district on the island of Oahu. It is a very aggressive parasite of pineapple roots during the winter months and causes, in the majority of cases, the death of the entire plant (23). Its differentiation from species of *Phytophthora* in the tissues of the host depends on the greater diameter of its hyphae, and the absence of fine ramifications at the tips of the hyphae (text fig. 22).



TEXT FIG. 21. *Pseudopythium phytophthoron.* Hyphae in root tissues (× 600).

Ph. Meadii MacRae and *Ph. Melongenae* Sawada

These organisms have been obtained often from the diseased roots of pineapple plants and from the soil of different fields on the islands of Oahu, Maui, Lanai, and Molokai. Both are parasites of roots, but more so of the stem, causing heart rot (23) during the winter months. They are inter- and intra-cellular parasites and may kill the tip or the entire root (text fig. 21).

Behavior of Fusarium Spp. in Pineapple Root Rot

There are many species of *Fusarium* associated with diseased pineapple roots. One of these occurring quite often was selected for these studies. This organism appears, from morphological evidence, to be related to *F. affine* Faut. et Lamb. (15, 36). According to Sherbakoff (14), "*Fusarium affine* can be at once distinguished from all the other Fusaria by its minute, one-septate, nearly straight conidia and its inconspicuous slow growth." The pineapple organism differs, however, from *F. affine* in that it produces



Text Fig. 22. *Phytophthora Meadii.* Hyphae in root tissues (× 300).

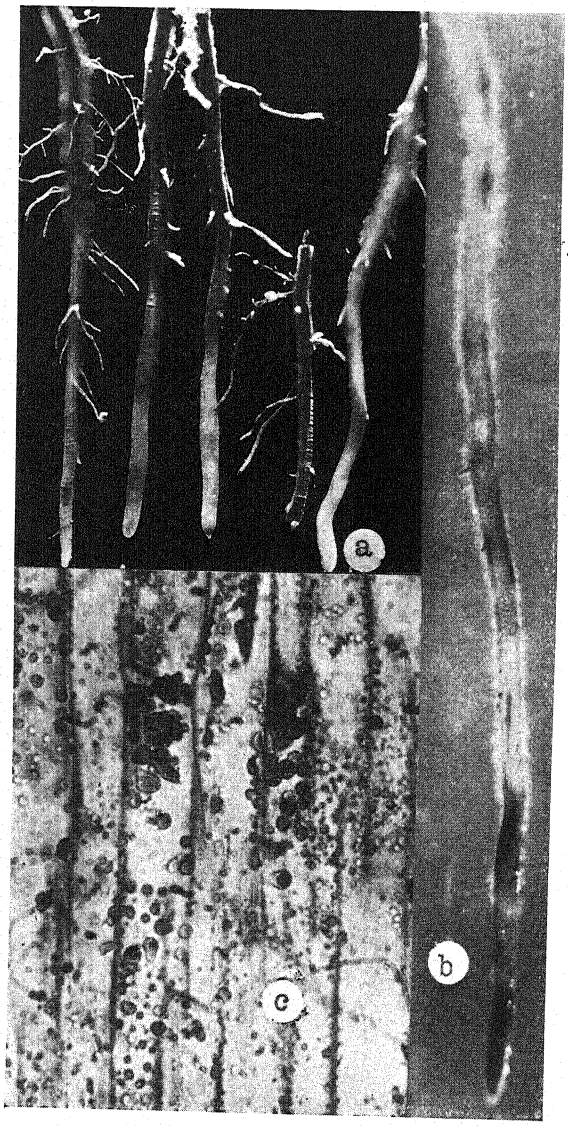
few two-septate conidia besides the one-septate and non-septate and also a moderate development of aërial mycelium.

Measurements of the conidia of the pineapple organism, grown in potato agar, are as follows:

0-septate	9 × 3 (7–12 × 2.0–3.0) μ
1-septate	11 × 3.5 (10–15 × 3.0–4.0) μ
2-septate	20 × 4.5 (18–25 × 3.5–4.5) μ

It is believed that the differences between the pineapple organism and *F. affine* are possibly varietal.

The results obtained from the various inoculations indicate that the pineapple *Fusarium* is a facultative root parasite. The roots which it attacks must have always some dead tissues for its initial saprophytic



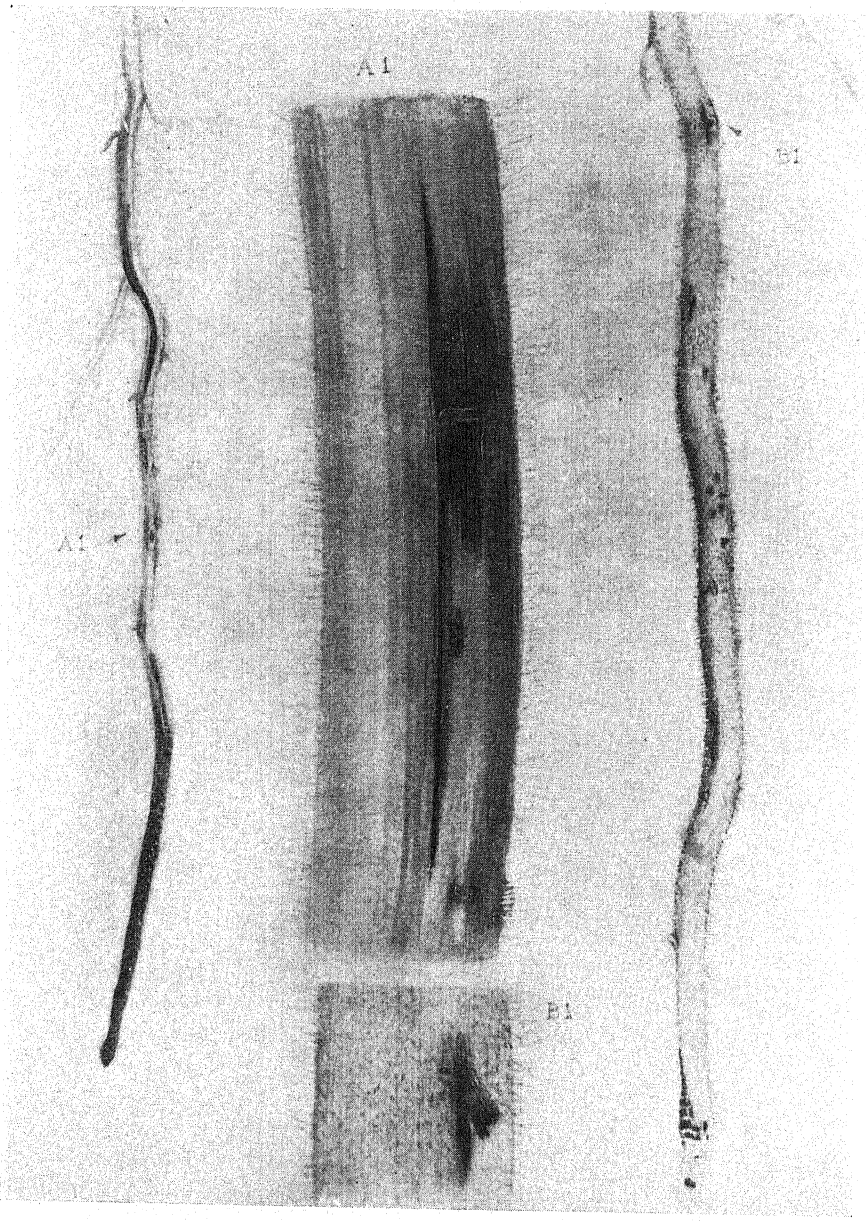
TEXT FIG. 23. *a*, *Fusarium* infected roots (four on the left diseased, one on the right healthy) showing brown discoloration at the point where laterals are developing. *b*, Roots grown in the soil and inoculated. Note the brown discolorations on the laterals. *c*, Cell contents of discolored tissues before final breakdown develops and invasion by the fungous hyphae takes place (× 600). Note the fat-like globules in the cells.

development. The further invasion of healthy tissues takes place after such tissues have been killed through the absorption of the toxic metabolic by-products of the fungus which are formed during the decomposition of the dead tissues (text fig. 24). Roots attacked by this organism die very slowly requiring from three to six weeks and possibly longer according to the number of initial infections. Few to many initial infections may start on the same root simultaneously (text fig. 23). They may be observed to increase in size through the discoloration of the tissues until they finally coalesce. Roots attacked lightly are able to recover usually by producing laterals above the diseased area, but severely attacked roots die.

It is difficult to assign any definite amount of the wilt disease (resulting through this type of pineapple root rot) to species of *Fusarium* on account of the inconsistent results obtained from inoculation studies. These organisms are responsible doubtless for some portion of the wilt disease most probably under drought conditions owing to the death through dehydration of certain weak laterals. It is likely that their association or presence with species of *Nematosporangium* and *Pythium* (judging from the results of few inoculations with mixed cultures) may result in a greater percentage of deaths of pineapple roots than otherwise. Most species of *Nematosporangium* and some of *Pythium* are very aggressive parasites of pineapple root tips, but they invade very seldom the highly lignified tissues of the root. It has been often observed that the dead tissues of roots, resulting through the initial parasitism of species of *Nematosporangium* and *Pythium* are gradually invaded by species of *Fusarium*. The latter organisms dominate the substratum in a short time, at the expense of the former, and are able to penetrate often into the healthy tissues of the root. Careful studies on dissected tissues of diseased roots proved that the tissues at the border line between healthy and diseased are occupied invariably by species of *Fusarium*.

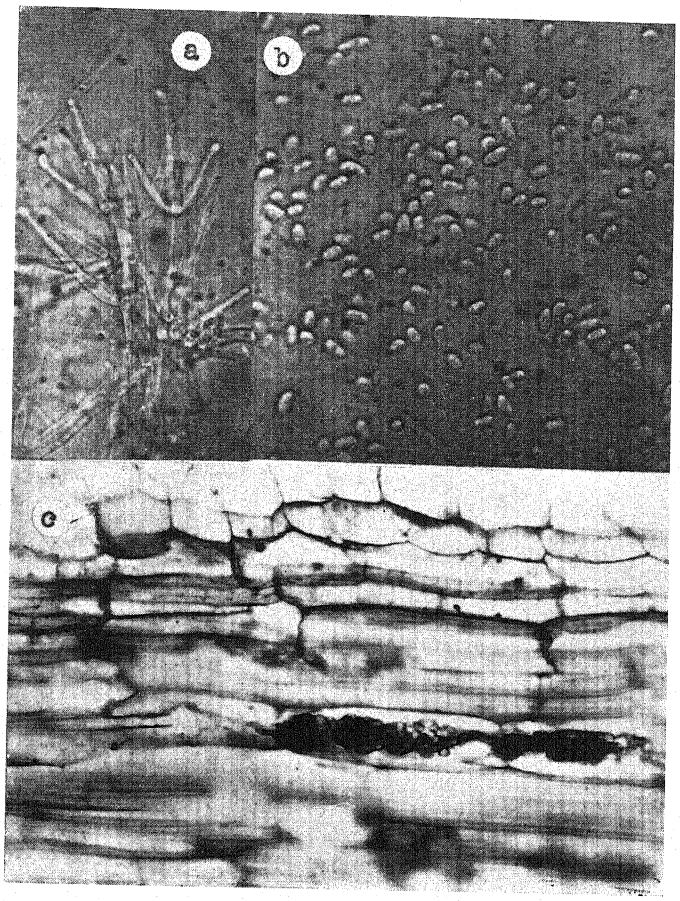
Behavior of Verticillium Spp. in Pineapple Root Rot

The presence of species of *Verticillium* in the diseased tissues of pineapple roots although not very frequent is by no means rare. In the course of these studies four different species were isolated. Of the four, only one proved to be pathogenic on inoculation. The pathogenic organism has been identified through the kindness of Dr. C. L. Shear as being closely related to *Verticillium heterocladum* Penzig (3). Penzig's organism occurs on the coccids of the leaves of orange trees. Another species of *Verticillium* which the pineapple organism closely resembles is *V. Buxi* Link (9). This organism, which is also a close relative of *V. heterocladum* Penzig, occurs on the tissues of withered leaves of *Buxus sempervirens*. It is difficult to state definitely, in the absence of authentic cultures, which of the two species of *Verticillium* is the closest relative of the pineapple organism.



TEXT FIG. 24. (From a painting by Mrs. A. M. Eller.) *A* (left), *Fusarium* infected root with brown discoloration (× 2); *B* (right), diseased root with brown discoloration (× 4); A_1 (center), longitudinal section of a position of root *A*, showing brown discoloration along the tissues neighboring a lateral and in the pericycle (× 25); B_1 (center), a superficial portion of root *B* showing brown discoloration (× 25).

Measurements of the conidia of the pineapple *Verticillium* give an average value of 5 × 2.5 (4–8 × 2–3) μ. These values correspond quite closely to those of *V. buxi* Link and *V. heterocladum* Penzig. The diameter of the main hypha of the conidiophore is about 3 μ and that of the branches



TEXT FIG. 25. *a* and *b*, conidiophores and conidia (× 600) of *Verticillium* sp *c*, diseased tissues of pineapple roots showing the parasite in one of the cells (× 300).

ranges between 1.8 and 3.2 (apex, 1.8 μ; middle 2.8–3.2 μ, and base 2.4 μ) (text fig. 25).

Examinations of diseased roots have shown that the parasite is capable of invading the parenchymatous tissues and water-conducting vessels causing their disintegration and death (text fig. 25). The initial entrance of the parasite into the tissues is made at a point 2 to 6 mm. above the root tip or through very young or weak laterals. The parasite grows in the tissues intra- and inter-cellularly. The cavities of invaded cells are occupied

either partly or totally by the pathogene. In the latter case, the hyphae of the fungus form massive bodies by doubling up, this condition resulting possibly through the mechanical resistance of the walls of certain cells to the penetration of the hyphae of the pathogene (text fig. 25). Infected roots may suffer either a partial or a total destruction of their tissues depending on the degree of resistance to the pectolytic and cytolytic enzyms of the fungus. The economic losses sustained through the parasitism of this organism are insignificant, however.

Discussion

Evidence has been presented in the foregoing pages to indicate that pineapple roots are susceptible to root rot caused by various organisms. The severity and symptoms of this malady vary not only with the organisms of the different genera but also with those of the same genus.

The different species of *Nematosporangium*, except *N. indigoferae*, are by comparison the most aggressive parasites of pineapple roots. The aggressiveness of the pathogenicity of these organisms, measured by the rate at which they invade and kill the tissues, is very high. They have been observed to kill the tips of roots to an extent of 2–4 mm. in 12 hours after inoculation. Such a process can be easily followed in root study boxes by a low power binocular, and its rate for each pathogene determined.

The succulent tissues of roots, either in naturally or artificially inoculated plants, die within a week in the majority of cases. Infected plants, if unable to produce new roots, often die, but those able to send out new adventitious roots or to produce laterals from the healthy tissues of diseased roots, survive. Resistance, in pineapples, may be interpreted as the ability of infected plants to produce new roots and thus perpetuate the life of the plant, and susceptibility as the converse of this condition.

The presence or absence of perfect oöspores and their relative numbers in the tissues of their hosts, which are characteristic features of the different species of *Nematosporangium*, bear a direct relationship to their distribution. It appears that under adverse conditions no other type of reproductive organ is as efficient as oöspores.

Pythium behaves much like *Nematosporangium* in the invasion and killing of roots except that its members are not as aggressive parasites as those of *Nematosporangium*. There is very great variation in the pathogenicity of species of *Pythium*, some of them being very aggressive parasites, others moderate or weak, and still others only saprophytes. Intravascular parasitism is more common with members of *Pythium* than with *Nematosporangium*.

An interesting development in these studies is the approximate (and in many cases relatively accurate) identification of different organisms in the tissues of their hosts on the basis of the morphology of their reproductive organs. The various illustrations explain the behavior of the different

species of *Nematosporangium* and *Pythium* in the tissues of the roots. As the production of oöspores by members of *Nematosporangium* and *Pythium* is influenced considerably by the amount and kind of food available in the tissues of hosts, it is possible that exceptions will occur especially in host plants with very thin (small diameter) roots.

Pseudopythium phytophthoron, *Phytophthora Meadii*, and *Ph. melongenae* are responsible for the development of root rot during certain seasons of the year, but not during the entire year, as is the case with species of *Nematosporangium* and *Pythium*.

Environmental factors play a very important part in the susceptibility and resistance of plants to disease. It was observed that an excess of moisture in the soil favors infection of pineapple roots by pythiaceous pathogenes. In our experience of isolating pythiaceous pathogenes from the soil of diseased fields by the method of natural infection of the roots of healthy plants, we found that a thorough wetting of the soil for a certain period was essential. A certain period is essential to allow for the germination and growth of the pathogene and also for its establishment on the tissues of the host. It is of great importance that conditions be maintained as favorable as possible for the parasite until it comes in contact with the tender tissues of the roots of the host. If, however, moisture conditions change before the root tip is reached by the pathogene, the latter is likely to decrease its rate of growth and assume, moreover, a rest stage.

The extent of such a period of favorable moisture conditions is influenced by the soil temperature, the relative population of the pathogenic organisms, the rate of growth of such pathogenes, their distance from the growing root tip, and the physical condition of the soil.

Verticillium sp. appears to be influenced by moisture in its invasion of pineapple roots in the same way as pythiaceous organisms.

With *Fusarium* sp. it has been observed that conditions likely to injure the roots of the host and leave behind some dead tissues are instrumental in favoring infection. It was observed, for instance, that some of the plants growing in root study boxes would often develop hard rot in those roots adhering to the glass of the box. Likewise, plants that had been watered irregularly and where the chances for root injury through dehydration were favorable, would develop this disease. The pathogenicity of *Fusarium* sp. cannot be compared either with that of pythiaceous organisms or with that of *Verticillium* sp., because of the difference in the type of parasitism involved in the two cases.

The other environmental factor involved for either favoring or inhibiting pathogenicity is the relative acidity or H-ion concentration (pH) of the soil solution. In certain studies conducted by the senior author (21, 17, 18) on the effect of soil pH to infection by different species of *Fusarium*, *Nematosporangium*, *Pythium*, *Pseudopythium*, and *Phytophthora*, it was found that pineapple roots are more resistant at pH values between 6.5 and 4.5 than

above, namely, pH 6.8 and 8.2. Schaffnit and Meyer-Hermann (13) have obtained similar results with *Pythium debarianum* on potatoes.

The H-ion concentration of the soil solution may influence pathogenicity either by its direct action or indirect action on the tissues of organisms. It is possible to change, by its direct action, the colloidal state of the protoplasm of either the host or the parasite or both and thus influence the permeability and metabolism of the cells.

There is no doubt but that the pH of the surrounding solution influences greatly the assimilation and metabolism of various organic substances by fungi. The senior author (19, 20) found in certain physiological studies on fungi that the rate of utilization of certain substances by various fungi depended to a great extent on the pH of the surrounding solution. He found, moreover, that different organic compounds are utilized better at certain pH values than at others. To the particular pH values at which these compounds were utilized best by *Fusarium cromyophthoron* and without the shifting of the initial pH of the solution by the secretion of either acids or bases he assigned the name "isometabolic point." Various other investigators (7, 30, 31, 35) studying the physiological behavior of different fungi observed a similar behavior, that is, they recognized an "isometabolic point" during the growth of their organisms. These and other investigations on the behavior of fungi and higher plants indicate that H-ion plays a very important rôle in plant metabolism. Whether the same conditions which influence the assimilation of organic food substances by fungi influence in a similar manner that of host tissues by pathogenic species remains to be answered by further studies.

The H-ion concentration of a culture solution influences, if in appreciable concentration, the hydrolysis of the tissues of both the host and parasite, besides its effects on the metabolic activities of both these organisms, as discussed above. It is possible, however, that the degree of hydrolysis, due to the concentration of either the H or OH ions, may with different organisms or under various conditions affect the host more than the parasite, or vice versa, due to chemical differences in the composition of their cell walls, and thus cause injuries which in the case of the host will increase its susceptibility to infection.

The H-ion concentration may cause through its indirect action conditions which may interfere with the normal development of plants. The solubility of various salts in the soil solution depends to a great extent on the H-ion concentration of the solution, as for instance the solubility of iron, phosphorus, sulfur, calcium, etc. With respect to iron, pineapple plants are very sensitive and their vigor of growth depends to a great extent on the availability of this element. It is possible, therefore, that the pH of the culture solution may favor or inhibit even indirectly pathogenicity either by its direct effect on the tissues of the host or on those of the parasite or by its action on the availability of the various nutrient elements of the soil solution.

Aside from the environmental conditions mentioned above which have been observed to play a certain part in the development of root rot, there are other conditions or tendencies, possibly hereditary, which influence the susceptibility of plants to infection. Such inherent tendencies have been observed by the senior author (24) in the parasitism of different monocotyledonous and dicotyledonous plants by various pythiaceous organisms. Although the exact mechanism of such tendencies is not well understood, it is believed, however, that it is due to differences in the chemical composition of the root tissues of hosts. The theory of mechanical pressure employed by the hyphae of parasitic organisms as a means for invading host tissues does not seem to be supported by the investigations of the senior author.

Comparative histological studies of diseased root tissues show that in the case of pythiaceous organisms and *Verticillium* sp. the tissues undergo softening and complete disintegration, whereas with *Fusarium* sp. such symptoms are lacking but instead hardening and browning of the tissues develops. The condition of the diseased tissues, whether exhibiting a soft rot or a hard rot, is caused by the action of the enzyms or toxins of the parasitic organisms. Pectinase and possibly cytase are responsible for the type of rot caused by pythiaceous organisms and *Verticillium*, and toxins for the brown discoloration caused by *Fusarium*. Pitman and Cruess (11) have shown in some of their studies on pectin hydrolysis by the pectinase of various fungi that *Pythium* spp. are some of the greatest producers of this enzym. *Verticillium* is considered by the same investigators a moderate producer and *Fusarium* a very poor producer of pectinase. Sideris (16) in his studies on the extracellular pectinase of *Fusarium cromyophthoron* found that the young and healthy colonies of this organism never secreted pectinase, but the old and possibly dead colonies of this organism released some. Our observations on the type of root rot and rate of its development caused by each one of the three different organisms agree quite well with the information of the above investigators, indicating, moreover, that the quantity of enzym produced by each one of these organisms bears a direct relationship to the amount of rot produced in a certain period.

Microscopic examinations of discolored diseased root tissues, caused by *Fusarium* sp., show that the brown pigment consists of fat-like brown globules enclosed in the dead cells (text fig. 23). Similar structures have been observed by other investigators in connection with certain other diseases. Dufrenoy (5) found them in the grains of wheat (*Triticum vulgare*) parasitized by *Bacillus tritici* and Beauverie (1) in the tissues of *Ranunculus* sp. parasitized by *Uromyces ficariae*. Both these investigators considered these structures as remnants of degenerated protoplasmic matter. Politis (12) observed the formation of tannins by the mitochondria of the cells of the leaves of *Euonymus* and *Vitis* parasitized by *Oidium*. As the chemical

nature of the fat-like structures in the pineapple roots was not investigated we do not know whether they represent degenerated protoplasmic matter or tanniferous substances.

Summary

The susceptibility of pineapple roots (*Ananas sativus*) to different species of *Nematosporangium*, *Pythium*, *Pseudopythium*, and *Phytophthora*, was found to vary between the different genera and also between the different species of the same genus. Nine species of *Nematosporangium*, eleven of *Pythium*, one of *Pseudopythium*, and two of *Phytophthora* were found pathogenic to pineapple roots, some of these being highly aggressive and others very weak parasites.

Of the different species of *Nematosporangium* included in these studies, *N. rhizophthoron* and *N. polyandron* are responsible for the greater portion of root rot attributable to this genus.

Species of *Pythium* are less pathogenic than those of *Nematosporangium* but are responsible for a fair portion of root rot. The most active species are *P. splendens*, *P. diameson* and to a small extent *P. artotrogus* and *P. irregularae*.

The amount of root rot caused by *Pseudopythium phytophthoron* and two species of *Phytophthora* is small and mostly restricted to winter months.

Approximate, and in certain cases accurate, identification of various pythiaceous organisms in the tissues of the pineapple roots is possible by employing the morphology of the reproductive organs as a basis for their differentiation.

Root rot caused by pythiaceous organisms is probably one of the important causes for the development of the disease of pineapple plants known as "wilt."

Species of *Fusarium* have been often suspected of a portion of the root rot disease associated with the wilt disease of pineapples. One of these organisms, a species closely related to *F. affine*, was found to be able to establish itself on devitalized or dead small laterals, kill the healthy tissues by means of toxic metabolic by-products, and then make its inward advance. Injured roots may recover if the injury is light or die if it is severe. Drought conditions responsible for the death or devitalization of laterals favor infection by this organism. The damage caused by this organism under field conditions has never been determined but it is likely to be of minor importance.

A *Verticillium* species closely related to *V. buxi* Link and *V. heterocladum* Penzig isolated from diseased roots of pineapples proved on inoculation to cause injury and death to the roots of this plant.

The losses resulting through the pathogenicity of this organism are low, judging from the rarity of its association with the diseased roots of pineapples. It is an intracellular parasite, invading the cortex and stele of the roots and causing their death.

The writers are indebted to Dr. A. L. Dean for reading the manuscript and for suggestions in the preparation of this paper.

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THE MORPHOLOGY AND CYTOLOGY OF *MONASCUS RUBER*

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INTRODUCTION

According to recent textbooks dealing with the fungi, there appears to be a general acceptance of the perithecial development in *Monascus* as interpreted by Schikorra (17) in his paper on *Monascus purpureus* Went and *Monascus X*. His views follow upon and are undoubtedly influenced by Claussen's work (4) on *Pyronema*, which appeared in 1907, although, in general, his studies corroborate those of Barker (1), which established the fungus as a true Ascomycete.

A survey of the literature has, however, stimulated a further inquiry into such questions as the nuclear behavior within the ascogonium, the development of the ascogenous hyphae, and the formation of the ascus. In order to make a detailed investigation of these phenomena, an examination of *Monascus ruber* van Tieghem was attempted, since quantities of material have been available from moulded maize starch and from maize silage. Cultures from these two sources were identified as separate strains of the same species, and were fully discussed in a previous paper (22).

From the time the genus was discovered in 1884 by van Tieghem (20) till 1903, when Barker published his results, the morphology of the fungus reflected the views of the van Tieghem-Brefeld (2) school, and thus included a lack of recognition of any sexual process associated with perithecial development and the formation of a sporangium whose protoplasmic contents divided up to form an indefinite number of spores. During this period the terms "sporangium" and "ascus" were used interchangeably and with some confusion, and it was some time before the significance of Harper's account (10) of the distinction between the method of spore formation in the two types of sacs was realized.

In 1895 Went (21) published the results of a careful study of *Monascus purpureus*, a species discovered by him in Java, where it is used largely for coloring rice. His figures of the ascogonial branch and covering filament

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bore a marked resemblance to the early stages in copulation figured by de Bary (7) for members of the Erysiphales, but although Went noted the similarity, the inconstancy in form of the covering filament prevented him from regarding it as an antheridial branch. The succeeding studies by Uyeda (19), Ikeno (11), Kuyper (13), and Dangeard (6), complicated the morphology of the fungus, and retained the view that associated hyphae preceding perithecial development did not have any sexual relation. In connection with this controversial point, Dangeard states: "Brefeld considère cette anastomose comme ètant d'ordre exclusivement végétatif, et son opinion est generalement partagée."

Barker's paper which appeared in 1903 showed, however, that a fertilization process analogous to those observed by Harper in *Sphaerotheca* and *Pyronema*, definitely occurred in *Monascus*, but that although there was a nuclear migration from the antheridium to the female organ, an actual fusion between male and female nuclei was not seen. Barker described the formation of ascogenous hyphae which were produced from the ascogonium. The hyphae eventually enlarged to form asci, in each of which eight ascospores were delimited. The disintegration of the ascus walls left a mass of spores lying free within the perithecium, which was a compact covering formed from a mass of sterile hyphae developed from the stalk below the ascogonium.

Ikeno's paper of the same year followed the previously accepted interpretation of *Monascus* as a Hemiascomycete, but his figures are of interest since he shows ascogenous hyphae in a binucleate condition lying within the perithecial covering. He used the term "cytoplasma-ballen" to describe these hyphae, and found that they divided into a number of spore mother cells, each of which produced a number of spores. These spore mother cells in his "Sporen-complex" were undoubtedly the asci observed by Barker.

A short paper published by Olive (14), supported Barker's conclusions, but suggested another possible rôle for the trichogyne, since he observed the ascogenous hyphae developing from this structure. He described a similar hollowed out central cell, but since his paper included no drawings, a considerable proportion of its value was lost.

It was probably largely due to Claussen's difficulty in interpreting Barker's results that Schikorra began his work upon two species of the same genus. For Claussen says: "Zelbst aus den nach Mikrotomschnitten gezeichneten Bildern Barker's kann ich mir kein völlig klares Bild von der Weiterentwickelung der Zentral Zelle (ascogonium) machen."

Schikorra selected *Monascus purpureus* Went and another form *Monascus X* found in discolored rice grains in a fermentation plant in Berlin. He used Barker's method of hanging drop cultures for studying the various changes occurring during perithecial development, but employed microtome preparations with more satisfactory results than those obtained by Barker.

In fresh material, he found an antheridium and later a female organ originating from the same hyphae, and later a trichogyne separated from the rest of the female branch by a septum. A fusion occurred between the tip of the trichogyne and the antheridium followed by a migration of nuclei from the male organ into the trichogyne. A temporary dissolution of the wall which limited the ascogonium allowed for the further passage of the male nuclei from the trichogyne into the ascogonium where the nuclei were seen to become associated in pairs. From this structure small papillae budded out, while enveloping sterile hyphae began to arise from the stalk below. The papillae grew out to form ascogenous hyphae, similar to those of *Pyronema*, but later crowding together to form a compact mass within the two-layered sterile covering or perithecium. The ascogenous hyphae contained pairs of associated nuclei, and were sparsely septate. A typical "crozier" developed at the ends of these hyphae, formed just as in *Pyronema*, by a conjugate division of two pairs of nuclei followed by a separation of a penultimate ascus by means of septa. Nuclear divisions occurred in each ascus, and finally eight ascospores were delimited. The ascus walls soon disappeared, leaving the spores free in the perithecial cavity, where a considerable amount of residual cytoplasmic material remained after the disintegration of the inner perithecial wall, used partly to provide nutriment for the developing asci.

Schikorra considered that the fungus possessed a life history similar to that of *Pyronema*, but since the asci were scattered irregularly throughout the perithecium and the ascospores liberated only by the decay of this covering, it was placed in the family Aspergillaceae, belonging to the order Plectascales.

Observations upon Monascus ruber

Methods

As previously mentioned the material used during this investigation was obtained from infected maize starch in a factory near Johannesburg, South Africa, and from a silage mould prevalent in Australia. Perithecia were found in vast numbers in plate cultures upon maize seedling extract agar, within a week of inoculation. Small portions cut out from plates were fixed in various solutions, including Flemming's weaker mixture, Merkel's fluid, Gilson's fixative, but the formol-acetic-alcohol combination (100 cc. 50% alcohol; 6.5 cc. formalin; 2.5 cc. acetic acid; 1.5 cc. glycerin), gave most satisfactory results. The blocks from a gross cultural growth of mycelium after being embedded and cut in the usual way, gave preparations of perithecia which were too scattered to be satisfactorily studied. The following procedure was then adopted: hanging drop cultures using nutrient agar were made at intervals during several days. The minute agar clumps were then carefully lifted from the cover-slips, and fixed in the 50% formol-acetic-alcohol combination. In the last dehydration stages, a trace of

Fast Green stain was added to the absolute alcohol, in order to make the mass visible in the paraffin block. In this way it was possible to obtain well oriented transverse sections of such masses, so many young fruiting bodies being present in each section that the numbering of a series was made possible. Perithecia tend to develop in zones, with the result that a single clump of mycelium would give sections of approximately the same age, those from cultures sixty-seven hours old showing excellent early post ascogonial stages in development, while those four days old contained asci and ascospores in the process of being formed. Sections cut at 4 μ and 5 μ gave clear views of the cytoplasmic structures, and more defined nuclei. On the whole, staining in Heidenhain's haematoxylin for eight hours, followed by a rapid immersion of the slides in a dilute solution of Fast Green (1 in 10 parts of a saturated solution in 95% alcohol), gave the best preparations. Flemming's triple stain was unsatisfactory, but a schedule which included immersion in safranin for $\frac{1}{2}$ to $\frac{3}{4}$ hour, washing in water, rapid staining in dilute Fast Green, followed by a momentary washing in 95%, absolute alcohol, clearing in clove oil and xylol, gave delicate green-violet cytoplasm, and bright red nucleoles. This method had the advantage of being rapid, and being more practicable for material fixed and stained in toto, for besides the microtome preparations, examination of whole mycelial mounts was of additional value, as also was the observation of living material in hanging drop culture. This latter method of study was facilitated by the use of an incubator which could be fixed on the stage of the microscope. Hanging drop slides could be placed in the cavity provided, and the temperature kept constant at 31° C. Acknowledgments are due to the Bacteriological Department of the University of Wisconsin for the loan of the apparatus.

Results

Germination of conidia and production of mycelial branches were observed, and copulating hyphae could be recognized within 62 hours. The antheridial branch developed and was later pushed to one side by the upgrowing female organ, which soon became strongly arched. An hour later, the tip of the female organ was bent over the antheridium, its edge being strikingly bright and refractive, while a dark area appeared in the antheridium around this region (Pl. XXXIX, fig. 3*a*). Within half an hour a septum appeared, cutting off the female organ from the rest of the hypha (fig. 3*b*), and gradually its apex in contact with the antheridium became less defined. A wall developed during the next hour, separating a terminal trichogyne from the rest of the female organ (fig. 3*c*). At this stage it was evident that a passage-way had been established between the trichogyne and the antheridium, the point of fusion appearing distinctly hyaline. A temporary dissolution of the septum separating the trichogyne and the rest of the female organ, as recorded by Schikorra, was not observed. On the other hand, a broad hyaline area appeared in the region of the

septum between the trichogyne and the ascogonium—this area, at different focal levels, gradually darkened giving the appearance of a deep groove. During the next two hours the ascogonium swelled to some extent, while vacuoles appeared in the apical portion of the antheridium and in the trichogyne just above the point of fusion with the male structure, and also in its central region (fig. 3*d*). As this vacuolization became more pronounced, a hyaline area appeared below the wall separating the ascogonium from the stalk, probably the beginning of sterile hyphal development, because in several other young fruiting bodies in the mycelium these hyphae had made their appearance (fig. 8).

Cytoplasmic structures and nuclei are not represented in figures 3 and 8, although a few rounded areas corresponding proportionately to nuclei, are indicated. Nuclear behavior is more satisfactorily discernible in fixed material.

From the study of a fruiting mycelium fixed in formol-acetic-alcohol, figures 1, 2, 4, 5, and 6 were drawn. As far as possible the appearance was of material seen in one plane, so that nuclei lying in close proximity were not clearly defined, since one nucleus often lay at a slightly different level from the neighboring one. Often, however, the hyaline portion of the nucleus was visible and drawn.

Vegetative hyphae are much branched and septate, each cell being multinucleate, and densely filled with cytoplasm when young but becoming more irregularly vacuolate as it matures.

Conidia, produced singly or in chains, are similarly multinucleate, with one or more centrally placed large vacuoles present. They vary from 7–16 μ in diameter, and are usually spherical or pyriform in shape. A conidium being formed at the apex of a branch is shown in figure 1 in order to indicate the multinucleate nature of both structures and the size of the nuclei.

In spite of repeated examination of nuclei present in the hyphal, conidial and young fruiting structures, it is not possible to detect any substance lying between the nucleole and the nuclear membrane. A nuclear reticulum was observed in certain parts of the hyphae, by both Barker and Schikorra, so that this failure to corroborate their statements is doubtless partly due to technical deficiencies. The nuclear membrane, however, is ill-defined and often lumpy, and often the boundary of the nucleole irregular. The suggestion that these lumps form part of a reticulum, of which the remaining threads are not visible, is supported by the fact that the large ascus nucleus has an irregular reticulum clearly discernible.

Barker, however, considered the nucleole when well defined and deeply stained as a nucleus undergoing division, the mass being an aggregate of chromosomes, having an irregular outline. He concluded that the nucleus was obviously in a resting condition only when observed to include a clearly defined reticulum. The constancy of the former nuclear condition, how-

ever, militates against this view, for all through the developmental phases the nucleus consists of a hyaline region around a heavy nucleolus. The nucleus having a reticulum observed by Barker, was, in all probability, the ascus nucleus.

Ikeno described each nucleus as containing an intensively staining body, the "chromoblast," with a clear area around it, and although its functional characters were not discussed, the term strongly suggests that the nucleole contained the chromatin material of the cell. The persistence of this nucleole in all the material examined by the writer, indicates that in part, at least, it is a chromatin-bearing structure, for in the later mitotic divisions in the ascus, the nucleole gives an extended, often dragged appearance, occasionally assuming a dumb-bell shape.

The vegetative hyphae range from 4 μ to 6 μ in width, but those branches which produce fruiting bodies are shorter and more slender. A hypha of this type has been drawn (fig. 2) to show an antheridial branch comprising an antheridium and a young terminal conidium, and below the antheridium a young female organ has originated, pushing the first formed structure to one side. The exact number of nuclei in the antheridium cannot definitely be ascertained. The cytoplasmic structures in these hyphae are characteristically dense, consisting apparently of a homogeneous alveolar mass. The further growth and curvature of the female organ are represented in figure 4, where again the antheridium is terminated by a young conidium. Two walls have appeared, one separating the stalk from the fruiting body, and the other a terminal trichogyne from the ascogonial cell, and it is probable that nuclear migration has occurred, since the antheridium is devoid of nuclei, whereas the somewhat swollen ascogonium is multinucleate, three pairs of associated nuclei being present, although the nuclei of only one pair lie in the same plane. An apparently odd nucleus lies to the right of the ascogonium, but its associate may be present below and not visible. It cannot, however, be stated with conviction that this associated nuclear condition is always clearly defined. In many cases a mass of nuclei appear to occupy most of the ascogonial area, and this crowded condition prevents any pairing from being obvious.

The next figure (fig. 5), illustrating similar structures, is included to indicate a complete fusion between the antheridium and the trichogyne. Here the antheridium is also devoid of nuclei, and contains large vacuoles, similar to those observed in fresh material of this stage. The nuclei which originally belonged to the trichogyne possibly do not take any part in the nuclear migration, for they appear to persist for a certain time (figs. 4 and 5), but are absent in succeeding stages (fig. 6). However, since no degenerate nuclei were observed in any mount of these structures, and since it is impossible to follow the migration of the nuclei in detail, it cannot be stated with certainty that the trichogyne nuclei do disappear. It is clear, however, that after the vacuolization of both antheridium and trichogyne,

nuclei are absent, although the organs remain turgid for some time. Figure 6 shows very well the ultimate collapse of the antheridium and lack of nuclei in both this organ and the trichogyne, and the swollen multinucleate condition of the ascogonium, which is slightly plasmolyzed. The magnification of this preparation is somewhat higher, with the result that the nuclei appear larger, both in the vegetative and in the ascogonial cells.

The suggestion of some change in the wall separating the trichogyne from the ascogonium after the passage had been established from the antheridium, derived from observation of living material, has to some extent been corroborated by the use of microtome preparations. The evidence indicates that a pore is formed in the wall, but since the structures are exceedingly small, a sectional view of the ascogonium showing a clearly defined opening analogous to that described by Harper for *Pyronema*, has never been obtained. Several preparations show an elongated light area on either side of the septum. In one of these two nuclei are lying in this area, one on each side of the wall (fig. 7*a*). If the focal length is slightly altered, the pathway appears continuous although the edge of the septum is still intact across the cell (fig. 7*b*). The pathway in this case does not appear in a median part of the wall, but in other young ascogonia the position is somewhat different since it may occur either in the center or towards the upper edge. On the whole it seems probable that a pore is formed in the septum, since the evidence seems to point to such a development, and since no indications of a temporarily dissolved wall were obtained, although it is possible that this stage has been missed. Before leaving the discussion of figure 7, it must be noticed that the nuclear migration has not been completed, since nuclei are still visible in the antheridium around the tip of the trichogyne, and since the ascogonium has not begun to enlarge.

The succeeding events have been followed from numerous sections of perithecia. The development of the sterile hyphae from the stalk below the ascogonium is very rapid, so that an enclosure of irregularly branched anastomosing cells protects the enlarging ascogonium and its related structures. Schikorra found two distinct perithecial layers formed, but the inner layer described by him belongs, as will be noticed from the accompanying figures, in reality, to part of the ascogenous hyphal system. In these early stages the covering hyphae are turgid, and filled with normal vacuolate protoplasmic contents, so that they are difficult to distinguish from the ascogenous hyphae, which fill up the interior and lie in close proximity to these sterile cells. The ascogonium does not swell to any considerable extent before ascogenous hyphae are developed from it. The formation of papillae from the periphery of the ascogonium, as described by Harper and Claussen for *Pyronema*, and as figured by Schikorra for *Monascus*, was not observed. Median vertical sections of mature ascogonia show conclusively that the ascogenous hyphae arise as segments near the base of the ascogonium, which become separated away by walls. These hyphal

segments grow up around the ascogonium, closely appressed to it, and enfold its dome-like upper region. In most sections one of these ascogenous hyphae, in advance of that developed from the opposite side, grows three quarters of the way around the ascogonium, and usually overlaps the tip of the second hypha. The identity of the ascogonium is retained for some time as a rounded structure in the center, but as other hyphae are segmented off from its base, and grow up between it and the first formed whorl, it cannot be distinguished. A further explanation of the loss of identity of the ascogonial cell, is that ascogenous branches grow rapidly, expanding to fill up the available space within the perithecial covering. This covering has also increased considerably by growth and stretching of the anastomosing cells, before the contents disintegrate leaving flattened walls forced together by pressure from the ascogenous hyphae within. The disintegrated material from the perithecial cells is visible in sections of this stage, and probably provides nutriment for the rapidly enlarging ascogenous hyphae. The remainder of the ascogonium becomes lighter in consistency as the hyphae develop, vacuoles appear in it, and eventually it is unrecognizable at the base of the perithecium. Each ascogenous hypha becomes divided into cells by means of cross walls, which are difficult to stain sharply. Associated nuclei are often observed in these hyphae, but in some cases two widely separated nuclei seem to lie in a cell. Each of these has possibly an associated nucleus not visible and a septum has not yet been formed to divide the cell into two, but the nuclei are not conspicuously paired or markedly outlined among the dense cytoplasmic contents, although the presence of them in the hyphae helps in separating these hyphae from those destined to form the perithecial wall.

Somewhat later the end cells of hyphae appear to swell considerably, and in each two nuclei are visible either in close proximity or in various degrees of fusion. These cells are ascus initials, and are often curved considerably, particularly in those perithecial cavities which are small. The figures illustrating this situation are similar to those given by Brooks for *Gnomonia erythrostoma*, in which the ascus may develop from the terminal cell, often curved over to a varying extent. It must be pointed out, however, that *Gnomonia* is a relatively highly evolved Ascomycete, whereas *Monascus*, as will be seen later from the discussion, possesses all the characters of a simple form. In *Gnomonia*, the "crozier" development of asci also occurs, consequently it is probable that the terminal somewhat curved ascus initial represents a persistence of the more primitive type such as is found in *Monascus*, and from which the more complicated "crozier" has been derived. Terminal asci are also known in *Plicaria*, *Erysiphe*, *Phyllactinia*, and other members of the Ascomycetes besides the Plectascales. Not only do the end cells of the ascogenous hyphae form asci, but the second and third cells behind the tip may develop similarly, so that a row of three asci result from a single overarching ascogenous hypha. The original

orientation assumed by the hyphae is still evident in these later stages after the "rectascous" asci have become separate structures. Fusion of the associated nuclei in the ascus initial results in a large, conspicuous nucleus, having a clearly defined nuclear membrane and a reticulum often apparently connected with or radiating from the heavily stained nucleolus.

A series of three nuclear divisions follows within each ascus, so that eight daughter nuclei are formed. During this time the ascus has increased considerably in size and often contains one or more central irregular vacuoles, which later seem to disappear, for the ascus during nuclear division is filled with cytoplasm, of a lighter consistency than that within the ascogenous hyphae. There are few division figures visible among the preparations, but in several asci the reticulum is lumped heavily to one side of the nuclear membrane. This has been interpreted as a synaptic stage, indicating that this first division of the ascus nucleus is a meiotic one. In only one case is a poorly defined spindle found with aggregated material in the center, but later stages in division are fairly frequent. Long thin spindles do occur, in the third nuclear division, between heavy lumps of chromatin material when the ascus is unusually broad, but more often the dividing nuclear portions are close together, sometimes apparently being pulled apart. The dragging appearance may be caused by the microtome knife, but the suggestion is permissible that the chromatin aggregate in the apparently persistent nucleole is divided in a normally mitotic fashion, but fixation and other difficulties render these obscure, particularly since spindles are probably too short or scanty to be observed. The first two nuclear divisions occur in a plane parallel to the long-axis of the ascus, the final division being in an oblique plane, so that eight nuclei fit most efficiently into the available space, with a considerable quantity of cytoplasm between each.

These observations upon the nuclear behavior in the ascus recall those of Juel (12) upon various species of *Endomyces*, where the ascus nucleus was larger and richer in chromatin substance than that typical of vegetative cells, which, however, contained a chromatin body besides the clearly defined nucleole. Prophases indicating heterotypic divisions were infrequent and not conclusive, although he found spindles connecting later stages. Small thickened rods at the ends of these minute nuclear figures were probably similar to those in the telophase of the third division figured by Dangeard previously for *Dipodascus albidus*.

The ascospores are formed in the manner typical of the Ascomycete group, although the details of spore delimitation are incomplete. Fibrous material appears near that portion of the nucleus which lies against the ascus wall. This material gradually envelopes both the nucleus and a small amount of cytoplasm, so that eight ascospore regions are eventually rounded off. A considerable cytoplasmic residue is apparent for a time, but as the wide, colorless, brightly refractive spore walls are formed, it becomes considerably reduced. The asci collapse, leaving a large indefinite number of ascospores free in the perithecial cavity.

Cultures of the starch mould strain produced perithecia of uniform size, although small, but in the silage material vast numbers of perithecia of varying size were developed. These latter tended to be large, and to form a greater number of ascogenous hyphae, so that sections of them were somewhat confused. For the final stages of spore delimitation, however, they were most satisfactory, particularly for illustrating the portions of asci enclosing ascospore nuclei which bear a superficial resemblance to a "crozier."

Proceeding to a consideration of the individual figures drawn from microtome preparations, the fact that sharply defined staining of nuclei, cross walls, and the boundaries of young ascogenous hyphae, lying in close proximity to each other and to the edge of the ascogonium, were difficult to obtain, accounts in part for the divergent interpretations of early perithecial development which have been published.

Ascogonia as large as the one drawn in figure 9 were rarely found, but this one is included to show the multinucleate organ, each nucleus not differing in size from nuclei present in any of the vegetative or copulating hyphae. The preparation is overstained, and for this reason it is highly probable that segments destined to form ascogenous hyphae may be separated by septa, which are not visible. A more heavily stained area occupies one side of the ascogonium, which is being covered by a sterile hypha and whose residual trichogyne is visible on the left-hand side. A clearer concept of the origin of ascogenous hyphae is obtained from median vertical sections of young perithecia (fig. 10). Two such hyphae filled with dense protoplasmic contents have arisen from near the base of the ascogonium as segments cut off from this organ. One of the hyphae has grown around the apex of the ascogonium and is closely appressed to it. In the most favorable plane for drawing only the hyaline parts of the nuclei belonging to the hyphae were visible. Enveloping sterile hyphae are drawn for the most part in outline only, except where it is intended to show the turgid condition of the cytoplasmic contents (Pl. XL, fig. 13), or existing nuclei (fig. 15). Usually the sections are not median; in these cases the ascogenous hyphae appear to surround the ascogonium completely (figs. 13, 16, 17). It is this type of section which led Schikorra to conclude that two distinct perithecial coverings are formed. In a number of sections additional rows of sterile hyphae partially overlap the first layer, and in older perithecia it is clear that these do not all fuse to form a single covering, so that parts of the perithecial wall appear to be two-layered. It is not a constant feature, however, whereas one layer is always completely formed to function as the protective envelope.

In figure 12 two sets of ascogenous hyphae have been derived from the ascogonium, whose upper region gives the appearance of an isolated central cell. Some of the septa in the ascogenous hyphae are not included because they could not be made out with certainty; on the other hand, the binucleate condition is later more defined.

The origin of the first ascogenous hypha is clear in figures 13 and 14, but in the former the wall separating it from the ascogonium is not yet visible. The path of the ascogenous hyphae and nuclear arrangement in the latter figure is confused owing to portions of other hyphae lying in a superposed position. Thin ascogenous hyphae and apparently isolated cells included in figures 16 and 17 are merely parts of ascogenous hyphae cut in various ways.

It is important to note the position of the ascogenous hyphae in these early stages, particularly in figures 12 and 13, for in their upward growth and orientation a gap is sometimes left a little to one side, where two hyphae do not actually touch. It is this gap which was interpreted by Barker as a hollowed out "nest," since he considered the whole set of structures to be one enlarged ascogonium, having failed to observe the limits of the hyphae or their septa. Further the oblique sections indicate clearly how the apparently encircling group of ascogenous hyphae could have been mistaken for a hollowed out central cell. In a number of instances (fig. 15), an appearance of invaginating hyphae would be given were the septa not visible since the largest of the enfolding ascogenous hyphae could then be interpreted as a hollowed out central cell.

In referring to the ascogonium, Barker mentions that "on rare occasions the central cell is a large more or less spherical cell filled with much vacuolated protoplasm," often with protuberances at the surface, or more usually a spherical body enclosing a round cavity into which the hyphae force their way. The protuberances are, in reality, the ends of ascogenous hyphae lying in close proximity to the ascogonium, and the vacuolated condition includes the narrow spaces between neighboring hyphae and between hyphae and ascogonium, for the protoplasm characterizing these structures is not vacuolate but always a dense homogeneous mass.

The identity of the central ascogonium is evident (fig. 18), until the rapid expansion of ascogenous hyphae and perithecial wall obliterate it entirely. Although the nuclei in the hyphae appear more definite, a paired condition such as is figured by Claussen for *Pyronema*, and described by Schikorra for *Monascus*, is not very evident at this stage. There is possibly a deficiency in nuclear attraction, although a little later when end cells are beginning to expand as ascus initials, the associated condition is quite clear.

The various overlapping ascogenous hyphae appear somewhat confused in many cases (fig. 19), but the drawing is included to show part of an ascus containing two ascospores in the process of being formed, while ascogenous hyphae overlying young asci occupy the upper portion of the perithecium. The asci here are terminal structures, but in the next perithecium (fig. 20) the large nucleus characterizing an ascus is present in both the end and second cells of the prominent hypha.

There is no evidence that sterile hyphae comparable with the paraphyses

of more highly organized Ascomycetes occur; all those isolated parts of cells in the various sections belong to functional hyphae. Frequently, however, the lowest cell of a hypha may not form an ascus, but it is usually small.

As nuclear fusion occurs in the asci the nucleolar material of each nucleus appears to increase in size, and since the fusion process is somewhat prolonged, various phases of it may be seen in a single perithecium (Pl. XL, fig. 21, and Pl. XLI, figs. 25, 26, 28). Young terminal asci often lie in a hooked position due to the method of growth of the hyphae to which they are attached, but as they develop the asci separate away, and then appear as large isolated cells. The succeeding phenomena in the ascus take place very rapidly, for in one perithecium an ascus containing a resting nucleus may lie next to one in which the spores are already formed. This may account in part for the paucity of stages in nuclear division. Figure 23 shows the situation described, with, however, an additional interest. Part of a mature ascus in the lower right-hand corner of the perithecium shows two ascospore nuclei. In the next focal plane lies a nucleus belonging to another spore, although it can be seen to lie apparently between them. Since the delimiting fibers can only be made out with difficulty, it is possible to miss them, in which case the structure resembles a "crozier" formation in a striking manner. A number of such parts of asci are illustrated in figure 33. In the center of the perithecium four nuclei belonging to spores lie in approximately the same plane, but in the group just below, one of the two nuclei, which are clearly defined, is overlain by another nucleus, resembling a pair of associated penultimate nuclei. It is from sections such as these that Schikorra obtained his ascus developmental stages apparently analogous to those of *Pyronema*.

The arched position of the ascogenous hyphae is often retained even after the asci have begun to separate from one another as in figure 24, where the asci differ slightly in age since in the terminal one the transparent ascospore walls are being formed, while in the second the fibers have recently separated spore masses away from a considerable residue of cytoplasm. The wall separating this ascus from the third containing a conspicuous nucleus is not clearly defined; in fact all the septa appear to consist of delicate material which is easily disorganized.

It has already been mentioned that nuclear divisions appear to occur rapidly, so that although it is possible to find a young ascus undergoing nuclear fusion lying next to one in which the large fused nucleus may be in a resting condition, or even contracted into a synaptic bundle preparatory to meiosis (fig. 26), the later stages of this division were not observed. Various phases of the next two divisions are visible but the dividing nuclei are represented by an apparently drawn out and segmented nucleolus (fig. 29), spindles occurring only between the daughter masses of nucleolar or chromatin material when the ascus is unusually wide. The fourth

chromatin mass at the end of the second spindle lies in the next focal plane (fig. 27). The oblique position of the spindles in this figure indicates the direction of the last division process, while the first two occur parallel with the long axis of the ascus (figs. 28, 30).

The method of spore formation which has been described is illustrated in figures 29, 31, and 32, the beginning of the process being particularly clear in the ascus in the right of the first perithecium. Figure 32 is drawn so as to show the total number of ascospores being formed—the colorless areas are the spore walls not completely separated from the surrounding masses of cytoplasm. Various parts of asci with developing ascospores and disintegrating ascus walls are visible in the perithecial sections illustrated in figures 33 and 34. Figure 33 has already been described in connection with Schikorra's illustration of the "crozier" method of ascus formation. The average diameter of the whole perithecium is 45 μ, the same size as a single spore of *Pyronema*.

Discussion

In reviewing Barker's work on *Monascus* certain of his figures bear a remarkable resemblance to those included in the present study, with, however, a different interpretation, particularly of the immediate post-ascogonial stages. The study of *M. ruber* has shown conclusively that the ascogenous hyphae arise in a manner analogous to that of *Aspergillus* (5) by growing up around the ascogonium and being in close proximity to it. In the further development of the ascogenous hyphae and the formation of the asci, his description and figures are scanty, since the asci illustrated are evidently fully mature for they are separate and even scattered in the perithecial cavity. The process of ascus formation is considered by him to be a simple one, being merely a rounding off of ascogenous hyphae. This conception has been fully verified by the present study, which includes intermediate stages which are of assistance in following the interpretation. Since Barker did not observe the septation of the ascogenous hyphae, it was not possible for him to find more than one ascus developed from a single hypha, such as is frequently the case. In Miss Dale's study of the ascus development in *Gymnoascus* (5), there appears to be a considerable similarity with *Monascus*, although she finds asci formed from the ends only of the ascogenous hyphae. Dangeard's description of the asci as being swellings of the ascogenous hyphae, which ultimately develop eight spores, has thus been confirmed for *Monascus ruber*, with, however, the additional observation that ascogenous hyphae are septate and one or more of the cells comprising these hyphae may form asci.

The main features of the perithecial development as described by Schikorra for *Monascus purpureus* and *Monascus X* are found to be true for *Monascus ruber*, but there are certain important aspects of the morphology in the latter which cannot be due to specific differences, but to interpretation of the phenomena observed. The early association of male and female

structures followed by an opening established to allow for migration of male nuclei, and later the nuclear arrangement in the ascogonium are similar in both studies, but there is no indication of a disappearance of the septum between the trichogyne and the ascogonium in *M. ruber*, though there is evidence of a passage formed, large enough to allow of a nuclear migration. The events succeeding the swelling of the ascogonium are not directly comparable to those in *Pyronema* since no apical papillae are budded out, but the ascogenous hyphae arise as segments of the ascogonium near its base. These hyphae grow up around the ascogonium, enfolding it. There may be two or more layers of these hyphae which become septate, each cell finally being clearly binucleate.

The most important point of divergence in interpretation lies in the formation of the asci, already discussed in some detail, since it affects the natural position of *Monascus* among the simpler Ascomycetes. The method of ascus formation in *Monascus ruber* by means of the enlargement of the end, second, or third cell of the ascogenous hypha, accompanied by a nuclear fusion, is a simple one. It could conceivably belong to a type from which the more highly organized Ascomycetes such as *Pyronema* could have been derived, particularly if the arched condition of the ascogenous hyphae became more pronounced to form a hooked structure, and nuclear relations were more specialized.

In comparison with such groups as the Erysiphales, where a compact many-layered perithecial wall is developed from the hyphae below the ascogonium, or related orders in which various kinds of ostioles exist to allow for the liberation of ascospores, *Monascus* is more primitive. Its scanty exterior investment which decays before the ascospores can be dispersed place it definitely among the simpler ascomycetes, as first suggested by Barker. Further, the nutritive relations of the higher forms, which possess a definite system of paraphyses, must be considered a secondary development, since more primitive types, as illustrated by *Monascus*, produce only structures which are destined to form ascospores within the perithecial cavity.

Barker discusses the similarity of ascus formation in *Monascus* and the Gymnoascaceae, but finds difficulty in reconciling the earlier perithecial developments in these forms, and further considers that the much enlarged ascogonial cell in *Monascus*, as interpreted by him, bears a greater resemblance to the Pezizales than to the Gymnoascaceae. Since, however, these points of comparison are based on misinterpreted structures, the obstacles to a position nearly related to the Gymnoascaceae can be removed, for he points out similarities such as the coiling of a female organ around a straight antheridium which occurs not only in *Monascus* but in many members of the Gymnoascaceae and also in *Penicillium*.

In considering the Plectascales as a whole, there are only a few cases recorded where a "crozier" development precedes ascus formation. One is de-

scribed for *Magnusia nitida* by Miss Satina (16), but it is not possible from her figures to make any direct comparison with analogous phenomena in *Monascus*. She finds ascogenous hyphae arising from a septate ascogonium, and developing to some extent before curving over. Conjugate nuclear divisions followed by separation of a penultimate ascus apparently occur in the usual way.

It is probable, however, that a simple method of ascus development is the rule among the members of the group, which apparently include types nearly related to ancestral forms from which along various lines the higher Ascomycetes have been evolved. Of these, *Monascus* resembles in all probability the ancestor of *Pyronema*-like forms.

The primitive nature of *Monascus* is again emphasized by Barker when he summarizes the want of specialization of the sexual organs, the inconstancy in form of the antheridial branch, which may often terminate in a conidium, and finally the origin of both male and female organs from the same hypha which involves the existence of male and female nuclei somewhat closely related.

By corroborating the work of Schikorra upon the early copulation processes in *Monascus*, it is now possible to cite one definitely established case among the Plectascales in which there is a nuclear migration from an antheridium, through a trichogyne into the ascogonium, where, however, the nuclei do not unite. Nuclear fusion is delayed until the ascus initial is formed.

Miss Dale records a nuclear migration from an antheridium to a female organ in *Gymnoascus Reesii*, so that it is probable that this fungus, and also *Magnusia nitida*, possess copulating phenomena similar to those described above for *Monascus*. In many other members of the order, particularly among the Aspergillaceae, the ascogenous hyphae develop from an apparently unfertilized ascogonium, although the further growth proceeds till finally asci and ascospores are formed, the latter eventually lying scattered within the perithecium, as in *Monascus*.

Summary

1. Using cultures from two strains of *Monascus ruber* van Tieghem it has been possible to follow the various stages in mycelium formation and the development of fruiting bodies.

2. Preceding formation of a perithecium, an antheridium arises at the apex of a lateral branch; an outgrowth from the same hypha just beneath the antheridial septum becomes the female organ. The two organs fuse and a passageway is formed; a trichogyne is separated from the rest of the female branch by a septum. Nuclear migration occurs from the antheridium to the trichogyne and from the trichogyne through an opening into the ascogonium, where nuclei do not fuse.

3. The ascogonium swells slightly and produces ascogenous hyphae as

outer segments near its base. These hyphae grow up around the ascogonium and lie close to its surface. A protective covering is formed by sterile hyphae arising from the stalk below the ascogonium.

4. The ascogenous hyphae become divided into cells, in which clearly defined associated nuclei are visible only in the later stages, although nuclei are present in these hyphae from the time of their origin.

5. Terminal, second, or third cells of ascogenous hyphae may form asci, in which nuclear fusion results in the formation of the large nucleus having a well defined nucleole and reticulum.

6. First nuclear division of the ascus appears to be a meiotic one, two succeeding divisions result in formation of eight daughter nuclei.

7. Eight ascospores are formed in each ascus by the delimiting action of fibrous material arising from that part of the nucleus which is nearest the wall of the ascus.

8. Ascus walls soon degenerate, leaving ascospores free in the perithecial cavity.

9. *Monascus* is considered to belong naturally among the Plectascales in the family Aspergillaceae.

Grateful acknowledgments are due to Professor E. M. Gilbert, for suggesting the problem and for kindly help and critical advice during the investigation.

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EXPLANATION OF PLATES

All drawings were made with the aid of a camera lucida. Figures 3 and 8 were drawn from fresh material; 1, 2, 4–6 from mycelia fixed and mounted in toto; the remainder from microtome sections.

A 2 mm. apochromatic Leitz lens was used with a × 12 compensating ocular for the figures magnified 1983 times, for the other figures an oil immersion 1/12 lens with a × 10 (× 1580) or a × 12 compensating.

PLATE XXXIX

FIG. 1. Single hypha terminating in a young conidium, showing multinucleate character. × 1983.

FIG. 2. Hypha bearing young female organ below antheridium, which is separated from a terminal conidium. × 2084.

FIG. 3. Various stages in the developing sexual apparatus. × 736. *a*, antheridium pushed aside by young female organ; *b*, female organ curving over antheridium and separated from the main hypha by a septum; *c*, female organ divided into ascogonium and trichogyne, the latter in contact with the antheridium; *d*, enlarging ascogonium, trichogyne fused with antheridium, which is separated from the apex by a septum.

FIG. 4. Sexual apparatus, showing trichogyne and antheridium in contact, ascogonial cell having five nuclei visible, but others present in lower plane. The apical region of the antheridial branch is developing into a conidium. × 2084.

FIG. 5. Branch bearing antheridium and trichogyne, in intimate contact, an ascogonial cell having two of its nuclei visible. × 2084.

FIG. 6. Enlarged multinucleate ascogonium, trichogyne fused with antheridium, which has collapsed and is almost empty. × 3300.

FIG. 7. *a*, antheridium in contact with trichogyne, an ascogonial cell, showing pathway in which lie two nuclei; *b*, the pathway of the above preparation, at a different focal level, the edge of the septum still present. × 1983.

FIG. 8. Early stages in perithecium formation, in which the ascogonium is being enveloped by sterile hyphae, arising from the stalk below. The trichogyne and antheridial branch comprising a sterile terminal region a conidium and an antheridium, are still visible. × 736.

FIG. 9. An almost median section of a multinucleate ascogonium, with trichogyne on left, and an enveloping sterile hypha. × 2084.

FIG. 10. Branch bearing ascogonium with two ascogenous hyphae closely appressed to it, and parts of two sterile hyphae. × 1580.

FIG. 11. Part of young perithecium showing ascogenous hyphae apparently encircling the ascogonium. × 1580.

PLATE XL

FIG. 12. Young perithecium, of which the sterile covering cells still contain turgid cytoplasmic contents, and enclosing three sets of ascogenous hyphae around the apical portion of the ascogonium. × 1580.

FIG. 13. Incomplete wall of young perithecium surrounding a clearly defined septate ascogenous hypha originated near the basal region of the ascogonium. × 1580.

FIG. 14. A similar perithecium in which more than one layer of ascogenous hyphae lie in a superposed position, around the ascogonium. × 1580.

FIG. 15. A perithecium in which nuclei are still visible in the sterile covering cells, and ascogenous hyphae are segmented off from the ascogonium, the remaining triangular portion of it lying in the center. × 1983.

FIGS. 16 AND 17. Oblique sections of perithecia, showing the wall almost complete, the ascogenous hyphae apparently encircling the ascogonium. × 1580.

FIG. 18. Two well-defined layers of ascogenous hyphae within the perithecial wall. × 1983.

FIG. 19. An enlarged perithecium having a complete wall surrounding a part of a mature ascus in which spores are delimited, and overlapping ascogenous hyphae, the cells of the lower ones being rounded to form asci. × 1983.

FIG. 20. A wall enclosing a well-defined septate ascogenous hypha, of which both the end and second cells have formed asci with conspicuous nuclei. Other young asci are present, one of them still in a binucleate condition, while two contain definitely associated nuclei. × 1983.

FIG. 21. Perithecium containing binucleate ascogenous hypha, three asci having associated nuclei in various stages of fusion, and one ascus with clearly defined nucleus, with nuclear reticulum around the nucleolus. × 2084.

FIG. 22. Branch bearing perithecium enclosing a young terminal ascus, and a second isolated one with large resting nucleus. × 2084.

FIG. 23. Ascus containing four visible ascospores being formed, part of a second ascus in similar condition, and a third with a large resting nucleus. × 1983.

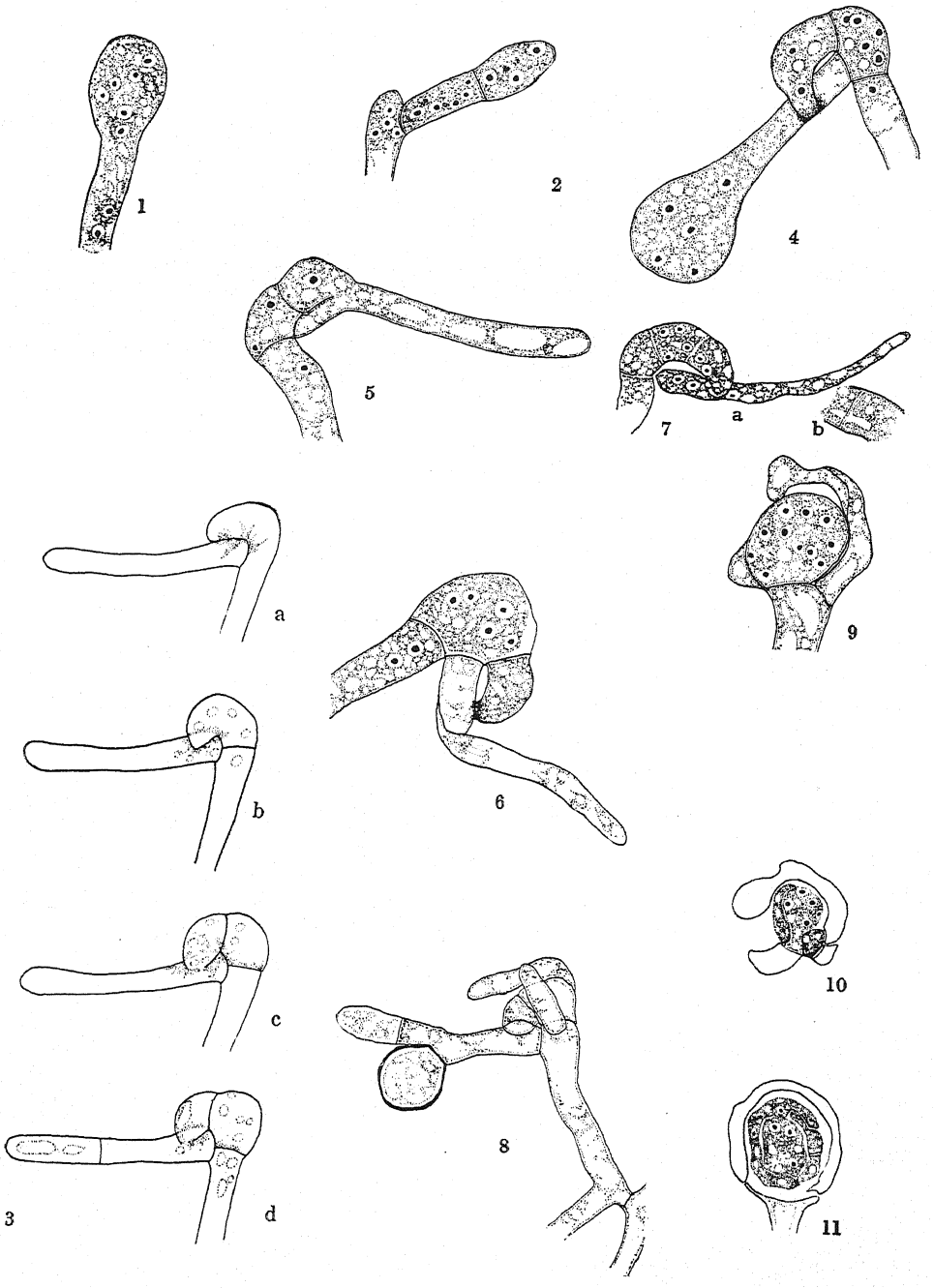
PLATE XLI

FIG. 24. Perithecium enclosing "rectascous" asci formed from one curved ascogenous hypha, end ascus with almost ripe ascospores, the second with ascospores being formed, the third with a single resting nucleus. × 1983.

FIG. 25. Young ascus, showing light vacuolate cytoplasm, and two almost fused nuclei. × 2084.

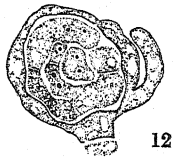
FIG. 26. Two asci showing nuclear fusion, and one in a synaptic condition preceding meiosis. × 1983.

FIG. 27. Two asci containing ripe ascospores and a third showing part of the last mitosis in the ascus, which is broad, so that slender spindles are visible between the daughter chromatin masses. × 1983.

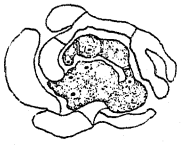


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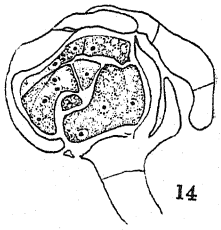




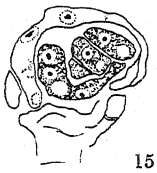
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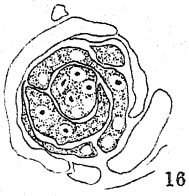
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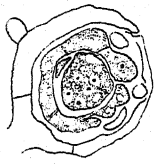
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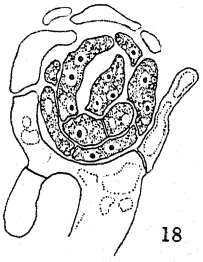
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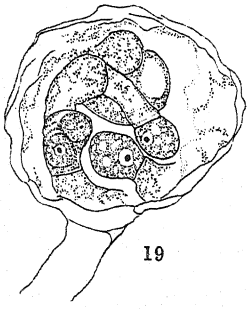
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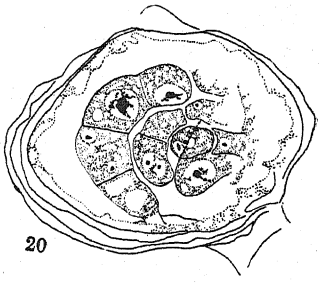
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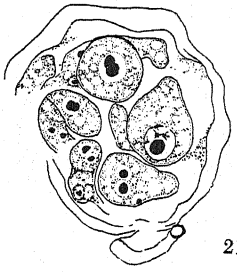
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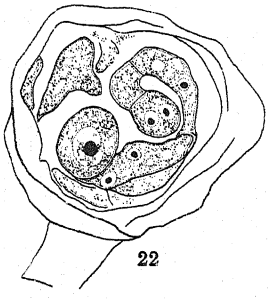
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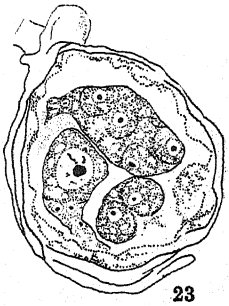
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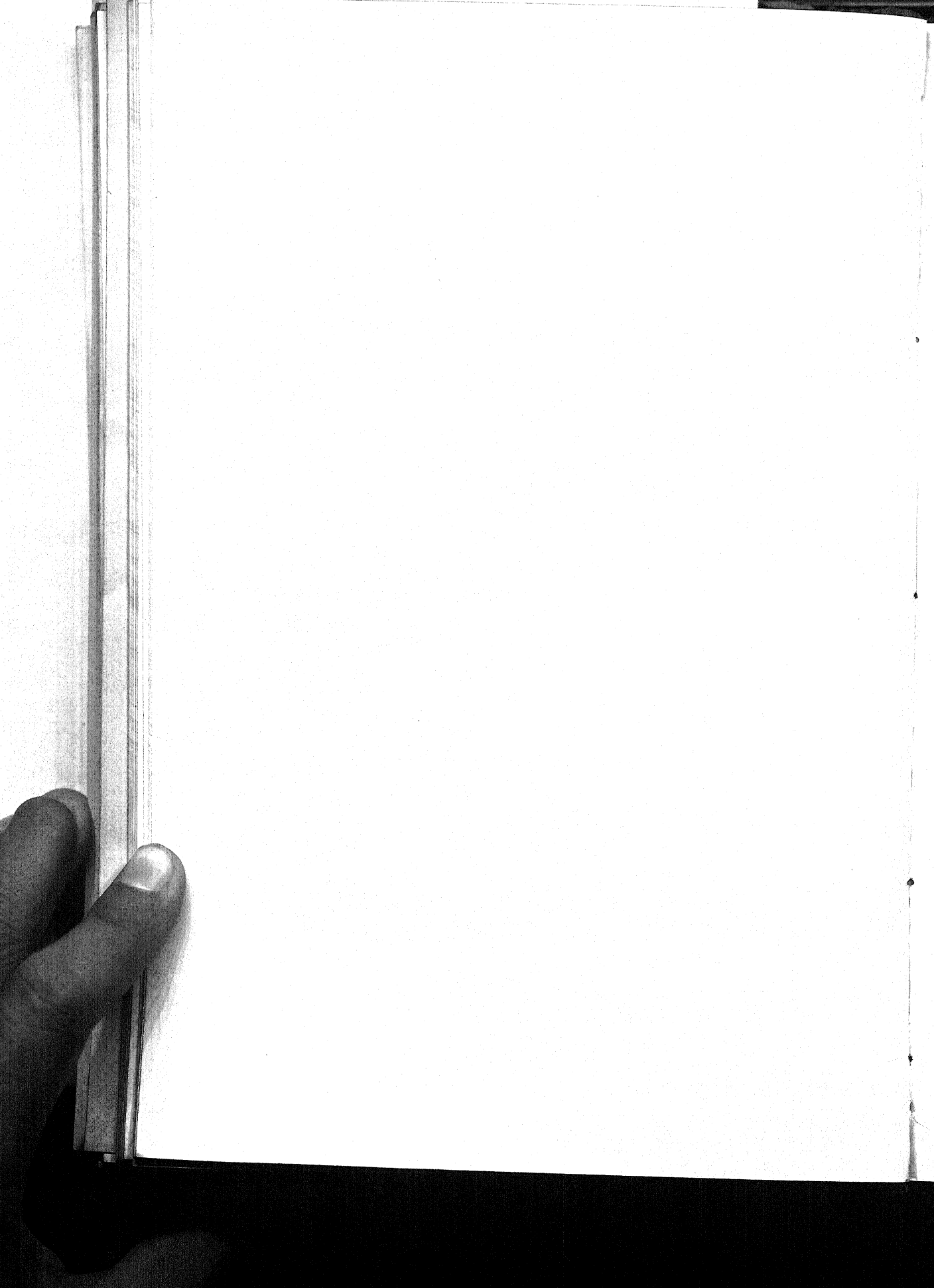


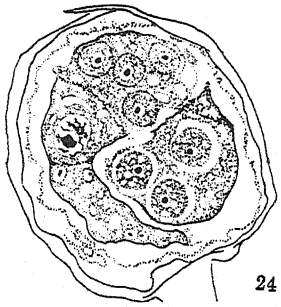
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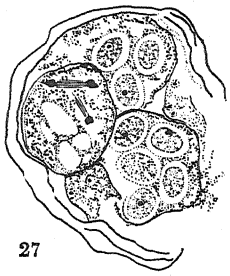
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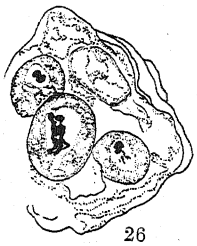
YOUNG: MONASCUS

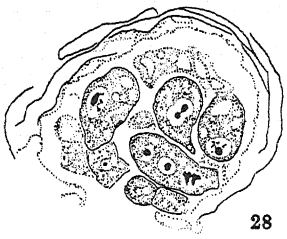


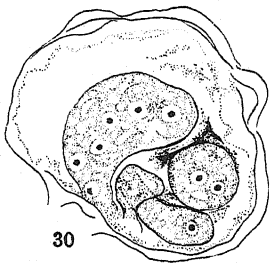


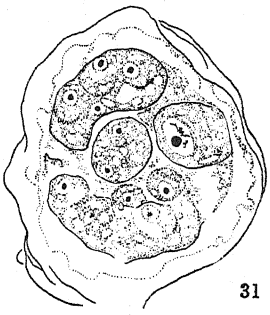


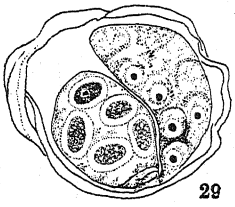




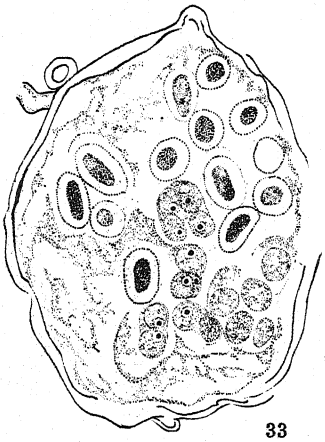


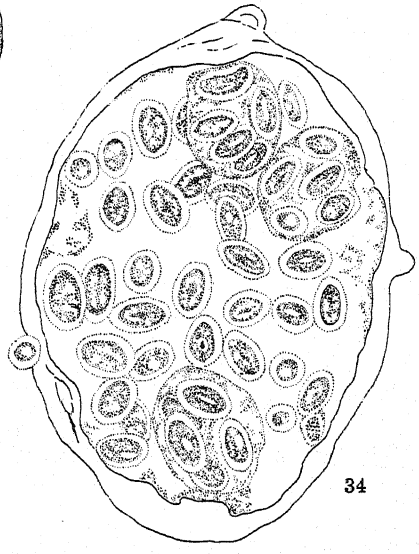












YOUNG: MONASCUS

FIG. 28. One ascus with nuclei fusing, two with resting nuclei and a fourth showing the second nuclear division being completed. × 1983.

FIG. 29. Two asci in a small perithecium—one showing fibers from near the wall of the ascus gradually surrounding nuclei and some cytoplasm, the other enclosing ripe ascospores and residual cytoplasm. × 2084.

FIG. 30. Wall enclosing large clear ascus containing 6 of the 8 ascospore nuclei, delimiting fibers beginning to appear; ascogenous branches and a young isolated binucleate ascus. × 1983.

FIG. 31. Perithecium containing portions of three asci in which ascospores are being cut out, and one ascus containing a resting nucleus. × 1983.

FIG. 32. Single ascus, drawn so as to show the portions of 8 ascospores, their colorless walls in the process of being formed.

FIGS. 33 AND 34. Almost mature perithecia: the former showing free ascospores, remains of ascus walls, and parts of asci in which groups of ascospores being formed resemble a "crozier" development (× 1580); the latter showing asci and ascospores lying free in the perithecial cavity (× 2084).

SOME PROPERTIES OF PLANT WAXES IN RELATION TO CLIMATE OF HABITAT

JAMES B. MCNAIR

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Since the melting points of fatty oils and alkaloids have been found to vary in accordance with the climatic habitats of the plants producing them, it is of interest to determine whether a similar condition exists in the case of vegetable waxes.

Waxes, like fatty oils, may be considered when chemically pure as salts of an acid or acids and an alcohol (esters). Waxes, too, have melting points intermediate between the oils and the alkaloids. If waxes have differences in melting points in accordance with their climatic habitats, then concomitant variations may be expected in their molecular weights and empirical formulae.

DATA AND DEFINITIONS

Data as to approximately 232 waxes have been assembled and reclassified for this paper from Grün and Halden's "Analyze der Fette und Wechse." Where data as to chemical constitution, name of substance, or melting point were not given in the above publication they were taken from the Smithsonian Physical Tables (1923). Molecular weights were either taken from "Van Nostrand's Chemical Annual" (1926) or were computed from the chemical formulae.

In the present paper waxes as obtained in their crude state from plants are defined as follows: Any of a class of plastic substances obtained from plants in greater or less purity, including carnauba wax and other compounds resembling beeswax in general properties and (more or less) in chemical composition. They are mixtures of compounds composed of the elements carbon, hydrogen, and oxygen. The chief proximate components of waxes are: (1) esters formed from higher fatty acids and mono- (or in some instances di-) hydroxy alcohols of high molecular weight, (2) free fatty acids, (3) free alcohols containing a high number of carbon atoms, (4) hydrocarbons of high molecular weight, and (5) ketones of high molecular weight such as myristone $(C_{13}H_{27})_2CO$, palmitone $(C_{15}H_{31})_2CO$, and β-cerotinone $(C_{26}H_{53})_2CO$.

In contradistinction to fats, chemically pure waxes may be considered as esters of mono- or di-hydric alcohols of high molecular weight, whereas fats are esters of trihydric low molecular alcohols, as glyceroi.

Climatic and Taxonomic Distribution of Waxes

In a previous publication (1929) the author has shown that of twenty plant waxes considered by Lewkowitsch four are found on plants growing in temperate climates and twelve are from tropical or subtropical plants.

According to the more recent compilation of Grün and Halden waxes are found in 84 plant families. These plant families can be climatically divided according to Engler and Gilg, and Willis. In this way, 32, or 38.09%, are mostly tropical; 4, or 4.76%, are mostly tropical-subtropical; 1, or 1.19%, is mostly subtropical; none is mostly subtropical-temperate; 17, or 20.23%, are mostly temperate, and 30, or 35.71%, are widely distributed in habitat.

The climatic distribution of wax families may be compared with the climatic distribution of the total number of plant families. Making use of the climatic distribution of these as determined from Engler and Gilg and Willis it becomes apparent that of the 295 families of gymnosperms and angiosperms 84, or 28.47%, contain wax. Of a total number of 153 tropical families 32, or 20.90%, have waxes; of 15 tropical-subtropical families 4, or 26.67%, have waxes: of 8 subtropical families 1, or 12.50%, has waxes; of 7 subtropical-temperate families none has waxes; of 50 temperate families 17, or 34.00%, have waxes, and of 57 families of widely distributed habitats 30, or 52.63%, have waxes.

It is thus evident that of the plant families producing waxes practically twice as many are found in the tropics as in the temperate zone. Those mostly tropical in distribution include 38% of all the wax families, while only 20.23% are found growing largely in the temperate zone.

The ratio of tropical wax families to the total tropical families (20.90%), however, is less than the ratio of temperate wax families to the total temperate families (34%).

The 32 tropical wax-producing families are: Palmae, Musaceae, Orchidaceae, Moraceae, Loranthaceae, Balanophoraceae, Aizoaceae, Anonaceae, Myristicaceae, Connaraceae, Erythroxylaceae, Simarubaceae, Meliaceae, Anacardiaceae, Balsaminaceae, Vitaceae, Tiliaceae, Bombacaceae, Guttiferae, Dipterocarpaceae, Bixaceae, Fouquieraceae, Araliaceae, Loganiaceae, Apocynaceae, Asclepiadaceae, Convolvulaceae, Verbenaceae, Bignoniaceae, Acanthaceae, Rubiaceae, and Cucurbitaceae.

The four tropical-subtropical wax-making families are: Amaryllidaceae, Iridaceae, Magnoliaceae, and Geraniaceae.

The only known subtropical family producing wax is the Hamamelidaceae.

No wax-producing family that is largely subtropical-temperate is known.

The 17 temperate wax-producing families are: Pinaceae, Juncaceae, Salicaceae, Juglandaceae, Betulaceae, Polygonaceae, Ranunculaceae, Berberidaceae, Papaveraceae, Resedaceae, Platanaceae, Empetraceae, Aceraceae, Umbelliferae, Primulaceae, Hydrophyllaceae, and Caprifoliaceae.

The wax families of widely distributed habitats of which there are 30 are: Gramineae, Liliaceae, Fagaceae, Ulmaceae, Aristolochiaceae, Rosaceae, Leguminosae, Linaceae, Rutaceae, Euphorbiaceae, Aquifoliaceae, Celastraceae, Hippocastanaceae, Rhamnaceae, Malvaceae, Violaceae, Thymeliaceae, Lythraceae, Oenotheraceae, Cornaceae, Ericaceae, Oleaceae, Styracaceae, Gentianaceae, Boraginaceae, Labiatae, Solanaceae, Scrophulariaceae, Dipsacaceae, and Compositae.

Physical Constants in Relation to Climatic Distribution

The compilations of the various physical constants of the acids, alcohols, hydrocarbons, and esters which have been found in vegetable waxes bring out some facts in regard to a probable relationship between climate of habitat and melting point, molecular weight, and empirical formulae of each of these chemical classes of substances (table 1).

Acids

The average melting points of the acids of the various climatic groups are seen to form an ascending series beginning with the tropical to the temperate and widely distributed. Those of widely distributed habitats should form a rough average between those of the tropical and the temperate habitats as such habitats include both tropical and temperate climates. It is thus apparent that the melting point-climatic ratios are not in accordance with but are the opposite of the melting point-climatic ratios of the oils and fats and of the alkaloids already determined by the author.

Of the various climatic groups of acids the highest melting points again show an ascending curve from tropical to temperate. If the minimum melting points be listed for the same groups there is formed a straight line through tropical, tropical-subtropical, temperate, and widely distributed.

Molecular weights would ordinarily be expected to vary, and in this case do vary, in accordance with variations in melting points. The highest, lowest, and average molecular weights of the various climatic aggregates agree in this respect.

The empirical formulae show a corresponding agreement in an ascending number of carbon, hydrogen, and oxygen atoms as from tropical to temperate sources in the cases of the average and largest formulae. The minimum formulae form a descending line in regard to carbon and hydrogen.

Alcohols

From the data at hand it is evident that ceryl alcohol is found in 7/9 of the temperate wax families and in 4/9 of the tropical wax families. Myricyl alcohol is found in 1/9 of the temperate and in 1/3 of the tropical; stigmasterin occurs in 2/9 of the temperate and in 1/9 of the tropical; and sitosterin in 2/9 of the temperate and in none of the tropical wax families. Citrullol, octadecyl alcohol, and daucosterin occur only in temperate

TABLE 1. *Physical and Chemical Constants of Wax Constituents in Relation to Climate of Habitat*

Habitat	Melting Point (° C.)			Molecular Weight			Empirical Formula								
							Carbon			Hydrogen			Oxygen		
	Max.	Min.	Av.	Max.	Min.	Av.	Max.	Min.	Av.	Max.	Min.	Av.	Max.	Min.	Av.
Hydrocarbons															
Tropical	68	37	62	436	282	374	31	20	26	64	42	53			
Temperate	75	62	67	507	324	418	36	23	29	74	48	60			
Widely distributed	75	30	61	493	324	414	35	23	28	72	48	60			
Alcohols															
Tropical	170	49	119	452	242	393	31	16	27	64	34	50	1	1	1
Tropical-subtropical	132	73	97	452	404	428	30	27	28	62	46	54	1	1	1
Temperate	283	75	131	724	270	401	30	18	25	62	38	42	1	1	2
Widely distributed	234	58	126	495	318	399	34	22	27	70	38	51	1	1	1
Acids															
Tropical	82	−18	38	410	228	279	26	16	18	52	32	34	2	2	2
Tropical-subtropical	82	−18	43	410	200	285	26	12	18	52	24	35	2	2	2
Temperate	281	−18	60	452	254	294	30	15	19	60	10	35	2	4	2
Widely distributed	130	−18	52	452	60	292	26	2	18	52	4	36	2	2	2
Esters															
Tropical	239	80	133	763	524	661	52	35	45	104	56	85	2	3	2
Temperate	84	79	81	761	621	696	52	42	46	104	84	93	2	2	2

families, while lupeol, loranthyl alcohol, and mesembrol are only found in tropical families.

The average highest and minimum melting points of the alcohols form an ascending series beginning with the tropical to the temperate and widely distributed. Those of widely distributed habitats should form a rough average between those of the tropical and temperate habitat. Because of the small amount of data obtainable for the tropical-subtropical group the figure given is not considered as dependable as the others. It is thus apparent that the melting point-climatic ratios are the opposite of those for the oils and fats and alkaloids already published by the author (1929, 1931) *i.e.*, tropical waxes have lower melting points than temperate waxes.

Molecular weights would ordinarily be expected to vary in accordance with variations in melting points, and form no exception to the rule in this instance.

The empirical formulae may ordinarily be expected to have corresponding variations in the melting points and molecular weights. The average and highest empirical formulae for the various climatic groups form descending curves from tropical to temperate although the difference between the terminal points is but small. The curve formed with the minimum formulae is ascending.

Hydrocarbons

If the highest, minimum, and average melting points be listed for the same groups there is formed an ascending line through tropical, temperate, and widely distributed.

The molecular weights would ordinarily be expected to vary in accordance with variations in melting points. The highest, minimum, and average molecular weights of the various climatic aggregates all form ascending values from tropical to temperate and widely distributed.

The relationships between wax hydrocarbons and climate in regard to empirical formulae harmonize with the variations in melting points and molecular weights.

Esters

The data on but twelve esters are available and consequently the relationships may not be reliable as criteria for the group. However, esters are influenced by the chemical and physical properties of the substances from which they are formed. As these alcohols and acids, as shown previously in this paper, have melting points, molecular weights, etc., which vary in accordance with the climates in which they are formed, esters therefore may be expected to vary similarly.

Discussion

The melting points, molecular weights, and empirical formulae of the hydrocarbon, acid, alcohol, and ester constituents of waxes indicate a variation in all of these values in accordance with the climatic habitat of their source plants.

In harmony with this relationship wax hydrocarbons, acids, and alcohols from the tropics have lower melting points, greater molecular weights, and larger empirical formulae than those of the temperate zone. The value of wax constituents from widely distributed habitats are intermediate between those of the tropical and temperate waxes, as might be expected since these habitats include both tropical and temperate climates.

This climatic-physical constant ratio is the opposite of those of the fatty oil and alkaloid, for the melting points and molecular weights of both fatty oils and alkaloids are greater when of tropical origin than when of temperate origin. It is of interest to note in this connection that the melting points and molecular weights of the wax constituents are higher than those of the fats and lower than those of the alkaloids.

Formation of Wax Hydrocarbons, Alcohols, and Acids in the Plant

For each climatic group (table 1) it is apparent that wax hydrocarbons have a larger number of carbon atoms in their molecules than either wax alcohols or wax acids. Wax alcohols have a greater number of atoms of carbon than wax acids. Similarly wax hydrocarbons contain larger numbers of hydrogen atoms than are present in either wax alcohols or wax acids. Wax alcohols have a larger number of atoms of hydrogen than wax acids.

No experiments seem to have been made to indicate the sequence in the development in the plant of wax hydrocarbons, alcohols, and acids. From the tabulated figures at hand, however, conjectures may be made. The lower number of hydrogen and the greater number of oxygen atoms in the wax acids than in the wax alcohols (as shown by the maximum, minimum, and average figures in table 1) indicate that the acids might be formed by the oxidation of their respective alcohols. This is also suggested by the melting points of the acids which are lower than those of the alcohols.

However, the fact that the molecular weights of the acids are less than those of the alcohols would indicate a splitting of the alcohol molecules if the acids are formed from them, because the addition of oxygen in acid formation from alcohols increases the molecular weight of the acid over that of its corresponding alcohol.

Although there are no available experimental results to indicate succession in the formation of hydrocarbons, alcohols, and acids in wax production it may be profitable to study the sequence of formation of these substances in the cases of fat and essential oil. It is well known that in fat formation the combining alcohols and acids are formed before their resultant esters. In essential oil development Charabot has shown a similar situation to exist in lavender and bergamot oil elaboration. It seems quite likely, therefore, that in wax formation the acids and alcohols are formed prior to the appearance of esters.

Experimental data has been compiled for hydrocarbon formation in the essential oils of caraway (Schimmel and Co., 1896), lavender and bergamot

(Charabot, 1899, 1900). The results, however, are not concordant, for in caraway oil the hydrocarbons (terpenes) are probably produced first, to be followed by the formation of oxygenated constituents (the ketone carvone); while in lavender and bergamot oils the hydrocarbon content (terpenes) increases with the maturity of the plant and the ripening of the fruit, respectively. In bergamot a part of the oxygenated portion of the oil (an alcohol linalool) is dehydrated to hydrocarbons (terpenes). According to these observations wax hydrocarbons could be formed from wax alcohols or wax alcohols could be formed from wax hydrocarbons.

Function

Most vegetable waxes seem to be produced by the integuments of the organism and not internally (as are fats and alkaloids), and their value to the plant is apparently to repel water or resist its action and prevent its ngress or egress. Whatever the function of wax may be to the plant it is perhaps of more value to tropical plants than to temperate ones, since practically twice as many wax producing plant families are found in the tropics as in the temperate zone.

Summary

Data of approximately 232 waxes have been reclassified for use in this paper to develop new relationships.

Plant waxes are considered to include as components higher fatty acids, mono- or di-hydroxy saturated alcohols of high molecular weight, esters formed from these, ketones, and hydrocarbons.

Waxes are found in 84 plant families, or 28% of all the higher plant families. Of these wax-producing families 38% are tropical, 5% tropical-subtropical, 1% subtropical, 20% temperate, and 36% widely distributed.

Of all tropical families 20% have waxes; of all tropical-subtropical families, 26%; of all temperate families, 34%; and of all widely distributed families, 52%.

The melting points, molecular weights, and empirical formulae of the acid, hydrocarbon, alcohol, and ester constituents of waxes indicate a variation in value in accordance with the climatic habitat of the source plants.

Wax hydrocarbons, acids, and alcohols from the tropics have lower melting points, greater molecular weights, and larger empirical formulae than those of the temperate zone.

The value of wax constituents from widely distributed habitats, which include both tropical and temperate climates, are intermediate between those of tropical and temperate waxes.

The corresponding values of tropical fats and alkaloids are higher than of temperate fats and alkaloids. This climatic variation is the opposite of that of waxes.

For each climatic group wax hydrocarbons have a larger number of both carbon and hydrogen atoms in their molecules than either wax alcohols or wax acids. Wax alcohols have a greater number of both carbon and hydrogen atoms than wax acids. Conjectures are made as to the sequence of formation of these constituents.

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STUDIES IN THE CHYTRIDIALES VI. THE OCCURRENCE AND LIFE HISTORY OF A NEW SPECIES OF *CLADOCHYTRIUM* IN CELLS OF *ERIOCAULON SEPTANGULARE*

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INTRODUCTION

In the late spring of 1929 a number of battery jar cultures of *Nitella flexilis* and *Chara delicatula* from Long Island, New York, were started in the laboratory, and among them grew up numerous plants of *Eriocaulon septangulare*, which had doubtless been growing with the Characeae in nature, and were collected and transplanted at the same time. As the summer progressed these plants grew very rapidly and thus gradually crowded out and displaced the stoneworts. During the fall and winter of the same year the leaves of many of them turned brown and finally became colorless. The leaf tips were first attacked, but eventually the entire leaves became involved. At this stage they usually broke away from the plant and rose to the surface of the water, so that it was no uncommon sight to find a large number of colorless leaves floating on the top of each culture.

Microscopic examination of such leaves revealed that they were infected by a species of *Cladochytrium* in the stage of numerous round and oval golden brown zoösporangia, opaque turbinate cells and a delicate mycelium-like rhizoidal system extending from one cell to another. In spite of the presence of this chytrid, however, the cultures of *Eriocaulon* have now been growing in the laboratory for more than two years, and so far this organism has not attained sufficient virulence to handicap the host plants very seriously. *Eriocaulon septangulare* seems to be an unreported and new host for *Cladochytrium*, and I have so far been unable to find any reference in the literature as to it as being parasitized by a chytrid. In view of this fact as well as the apparent ubiquity of this species of *Cladochytrium* I am reporting its morphology and life history as I have observed it. The data here presented relate only to living material. A comparative morphological and cytological study as well as cross inoculation experiments with all available species of *Cladochytrium* is now under way to determine their relationships as well as host range.

GENERAL MORPHOLOGY AND LIFE HISTORY

Vegetative Thallus

In badly infected leaves of *Eriocaulon* one may often find many stages of development of this chytrid within a small region of observation. With

the object of showing general distribution and habit of growth of this *Cladochytrium* in relation to the host cells, I have drawn (Pl. XLII, fig. 1) a portion of a leaf in which the fungus was unusually abundant. In this figure are shown numerous round, oval, and irregularly-shaped zoösporangia, zoöspores, so-called turbinate cells, and rhizoids. The vegetative portion of the thallus consists of a mycelium-like rhizoidal system and turbinate cells. The rhizoids or what may be appropriately termed "rhizo-mycelium" are very delicate and frequently quite colorless, and hence usually difficult to differentiate among the hyaline host cell walls, masses of degenerating plastids, and starch grains. For this reason I have in figure 1 somewhat accentuated its appearance in order to bring out more clearly its distribution and ramification in the host tissue, but have not attempted to show in this figure the manner of penetration of the host cell wall. As it passes through the hyaline cell wall the delicate rhizo-mycelium, in living material, is so similar in refractive index that it is almost impossible to determine the relation between the two. In a few exceptional mounts, however, the rhizo-mycelium has appeared notably constricted in the region that lay in the cell wall. I am leaving this problem for further study on fixed and stained preparations. A more typical picture of the rhizo-mycelium is shown in figure 4, Plate XLIII. It is variable in diameter, .5 to 3 μ, non-septate, branched and extends from one host cell to another, and as is well illustrated in figure 4, the ends run out to a marked fineness.

This rhizo-mycelium is thus somewhat different from the rhizoidal system in the Rhizidiaceae. In this latter family, as I have noted before (1930), each sporangium with its rhizoids is a separate and independent thallus usually confined to a single host cell, and the center of gravity of the growth process remains in the sporangium. In the Cladochytriaceae, on the other hand, the thallus is replicated to a certain extent as it spreads from one cell to another, and the center of growth is thus carried to new points where additional sporangia and spores are formed.

In view of these differences I have in contradistinction designated the rhizoidal or mycelial system of the Cladochytriaceae as a *rhizo-mycelium*. I do not, however, use this term in a phylogenetic sense to mean that such a system is primitive and has given rise to the mycelium of the higher fungi. I use it chiefly with the view of calling particular attention to the differences that exist in the vegetative thallus of the various chytrids. Most mycologists make no distinction between the rhizoids of the chytrids and the mycelium of the higher fungi, and it is not certain from their descriptions whether they use the term mycelium in a purely anatomical sense or phylogenetically. It seems to me that essential differences do exist and should be recognized. While the evidence is not very convincing, it may be that the rhizo-mycelium of the Cladochytriaceae is a transition type between rhizoids and mycelia.

At various intervals in the rhizo-mycelium occur the so-called turbinate

cells and zoösporangia. In view of the part these structures have played in classification I shall deal with each separately and in detail.

Turbinate Organs

The so-called "turbinate cells" (Jones and Drechsler), "Sammelzellen" (Büsgen), "Anhangzellen" (Fisher), "corps central" (Cornu), "vesicles collectives" (Vuillemin), "Antheridien" (Magnus), "companion cells" (Butler, E. J.), or "collecting cells" (Bartlett), constitute one of the controversial structural features of the Cladochytriaceae. These cells were first figured and described for the genus *Cladochytrium* by Nowakowski in 1877. Previous and subsequent workers, Wallroth (1833), De Bary (1864), Schroeter (1889), Tisdale (1919), Jones and Drechsler (1920), Bartlett (1926), etc., have figured and described them for *Physoderma* and *Urophlyctis*. Since they usually occur in pairs in many species and are frequently attached directly to the resting spores Schröter (1882–1889) regarded them as gametes whose contents fused, and hence he established the genus *Urophylctis* of the family Zygochytriaceae to include all species whose resting spores are formed by the fusion of two such similar cells. This view was supported by Magnus in 1897, 1901, and 1902, but has since been questioned, because turbinate cells may occur in various positions, quite apart from the resting spores (Nowakowski 1877, fig. 6, Pl. 6). Except in instances where one of the cells gives rise directly to a zoösporangium they are at the present time regarded as more or less modified portions of the vegetative thallus.

Since they appear to begin as spindle-shaped enlargements of the rhizomycelium which undergo division to become multicellular and whose cells then remain in close continuity, it seems to me that it is more appropriate to speak of the entire structures as turbinate organs rather than cells. In this sense the turbinate organ may consist of one or many turbinate cells. In the species of *Cladochytrium* which I have studied they are very abundant as more or less hyaline vesicles which may be composed of from one to six cells. A number of these turbinate organs are shown in figure 1, Plate XLII; and in figures 14 to 19, Plate XLIII, I have illustrated the variations in their size, shape, and contents as they have been observed in leaves of *E. septangulare*. Table 1 shows the variation in the number of cells which may compose these turbinate organs.

TABLE 1. *The Variation in the Number of Cells which Compose the Turbinate Organs from Leaves of Eriocaulon septangulare*

Type of Turbinate Organ	1-celled	2-celled	3-celled	4-celled	5-celled	6-celled
Number counted from leaves of *E. septangulare*	10	60	18	8	2	2
Percentage	10%	60%	18%	8%	2%	2%

It is obvious from this table that the largest number are two-celled, and that five- and six-celled types are rather exceptional. Similar variations in number of cells in the turbinate body have been reported for other species of *Cladochytrium*, *Physoderma*, and *Urophlyctis*. In *Cl. tenue* (Nowakowski 1871, fig. 6) they are 2-celled; in *Ph. Butomi*, *Ph. Alismatis*, and *Ph. Menyanthis* (Büsgen 1887, Ludi 1901, and Clinton 1902) 1- to 4-celled; in *Ph. Zeae-Maydis* (Tisdale 1919) 2- to 5-celled, in *Urophlyctis alfalfae* (Jones and Drechsler 1920) usually more than 4-celled, etc.

Quite often all of the cells may appear empty (figs. 1, Pl. XLII, and 15, Pl. XLIII). In other cases one of the cells may be filled with opaque globules or bodies (fig. 17, Pl. XLIII); while in some all of the cells are filled. Nowakowski, E. J. Butler, and Matthews have figured one of the cells in *Cl. tenue*, *Nowakowskiella elegans*, and *Now. ramosa* as becoming very large and subsequently being transformed into a zoösporangium. I have so far found only a limited number of such cases in the species which I have studied. In figure 18, Plate XLIII, is shown a turbinate organ composed of six cells, the terminal one of which is relatively large and filled with round bodies. Its extremity is extended into a long beak which resembles the neck of a zoösporangium. As noted before, it is not uncommon to find several of the cells filled with round bodies, but whether or not these are zoöspores has not been ascertained. They are markedly different in appearance from the zoöspores which I shall describe later.

According to Nowakowski's drawings the turbinate structure appears to begin as a spindle-shaped enlargement or swelling on the rhizo-mycelium. As it elongates and increases in diameter it apparently undergoes division once to several times, thus forming a multicellular organ. The division of the mother cell may be very unequal, as is frequently the case in *Physoderma* and *Urophlyctis* (Tisdale, Jones and Drechsler, and Bartlett). The position of the turbinate organs relative to the zoösporangia and resting spores varies considerably. In *Cl. tenue* (Nowakowski, figs. 6, 8, 9, and 10, Pl. 6) they may occur at widely separated intervals on the rhizo-mycelium or one cell may give rise to the zoösporangium directly while the other remains as an apophysis. In the so-called *Physoderma* and *Urophlyctis* species they occur relatively close to the resting spores. This is particularly true of *U. alfalfae* and *U. Potteri* in which it has been shown by cytological studies (Jones and Drechsler and Bartlett) that the resting spores bud out from one of the cells of the turbinate organs.

Development and Structure of Sporangium

The zoösporangium whether terminal or intercalary appears to begin as a swelling or enlargement of the rhizo-mycelium. In this species on *E. septangulare* the majority of sporangia observed have been terminal. In figure 1 *a*, *b*, *c*, and *d*, are shown a number of such enlargements which are early stages in development. Figures 2, 3, 4, and 5, Plate XLIII, show

further successive stages. In figures 2 and 3 the incipient sporangia are markedly club-shaped and not extended into a beak as in figure 1 *a*, *b*, *c*, *d*, and 4. At these early stages the cytoplasm of the sporangium begins to take on a characteristic golden brown color. At first the cytoplasm is grey, then pale orange or brown and finally becoming brilliantly golden brown. This color is so characteristic that the sporangia may be detected with great readiness before they are of mature size. It appears to be distributed throughout the cytoplasm in the form of minute granules or droplets, as Zopf has shown in the incipient sporangia of *Cl. polystomum*.

The shape of the sporangium generally becomes more spherical as development continues (fig. 3, Pl. XLIII). In the very large ones, however, the shape is not always spherical but seems to be largely determined by the confines of the host cell, as is well illustrated in figure 1 *e*. The sporangium here shown has apparently burst through the cell walls of the host into four additional cells. The intercalary sporangia, which have been very few in number, appear to be more oval than spherical in shape.

With the growth and increase in size of the sporangium the golden brown granules or droplets increase in size (figs. 1 *f*, *g*, *h*, *i*, *j*, and 3, Pls. 1 and 2). This change, with the formation of additional ones, suggests that the smaller droplets fuse to form larger ones, since in the fully mature sporangium globules of a more or less uniform size are present.

The size of the sporangium varies in the same degree as does the shape. Spherical sporangia at the time of cleavage vary from 8 to 18 μ in diameter. The general average of 50 such sporangia is 14.5 μ. As to the large irregular ones, the size and relative dimensions vary to such an extreme degree that I shall devote special attention to them later.

The sporangial necks begin to form very early (figs. 1 *i*, *g*, and 3, Pls. XLII and XLIII), as small papillae on the surface of the sporangium. Figure 5 shows an early stage in which the neck is approximately half the diameter of the sporangium in length. In this species of *Cladochytrium* the number of necks is predominantly one, but occasionally more than one may be observed, which is much in contrast with *Cl. polystomum* in which Zopf figures several to each sporangium. The necks may penetrate to the outside of the host or extend for a considerable distance through several cells without emerging. Numerous necks have been found which were more than 60 μ long.

As the sporangium matures it becomes invested with a distinct wall which delimits it from the remainder of the thallus (figs. 5 and 6, Pl. XLIII); and just prior to cleavage the ground substance of the cytoplasm appears optically homogeneous with a large number of dispersed or suspended highly refractive, golden-brown bodies or drops. These bodies are quite fluid and may be made to flow together in large irregular masses by severe pressure on the cover glass. The host cells at this stage also contain numerous round, oval, and irregular bodies (fig. 1), which are very similar to the ones in the

sporangia in shape and color, and at first glance one gets the impression that they are the same and have actually been engulfed by the fungus. However, a careful study of the development stages shows that this is not the case. Each zoöspore includes a single one of these globules. Because of the optical homogeneity of the cytoplasm as well as the opacity of the host cell it is difficult to follow the stages of cleavage in living material. Eventually the sporangium is filled with more or less hexagonal segments (fig. 1 *k*), in the center of which lies a single highly refractive brown globule.

Shortly thereafter the swarm spores usually escape or slip out *en masse* and lie quiescent for a few seconds at the mouth of the sporangial neck (fig. 1 *L*, Pl. XLII, and fig. 7, Pl. XLIII). Occasionally they may show some activity within the sporangium and swim out one by one. The resting period immediately following escape is also characteristic of *Cl. tenue* and *Now. elegans* according to Nowakowski. It is difficult to determine whether or not the zoöspores escape in a vesicle which shortly disintegrates, but the fact that one or more swarm spores remain behind in the sporangium (figs. 7 and 11), or escape separately suggests indirectly that no vesicle is present. Furthermore, escape of swarm spores in a vesicle has not yet been reported for any species of *Cladochytrium*.

Occasionally proliferating sporangia may be found. In figure 13 is shown a round, empty sporangium through which a second oval one is growing. The neck of the secondary is emerging through the neck of the primary one in a manner suggestive of what Nowakowski and Clinton have described in *Now. elegans* and *Ph. Alismatis*. In the latter species according to Clinton as many as five successive sporangia may be formed within the primary one. So far I have found only secondary sporangia.

Irregular Sporangia

Large and irregularly-shaped sporangia have been found in such abundance in dead host tissue that they merit special attention. Similar structures have been reported and figured by Clinton for *Ph. Alismatis*, Schröter for *U. pulposum*, and Matthews (1928) for *Nowakowskiella elegans*. In the first two species, however, they are formed on or very close to the surface of the host plant. In Plate XLIV there are shown several such sporangia from three host plants. Figures 20, 21, and 22 are from *E. septangulare* and *E. compressum*, respectively; figures 23 and 24 are from *Myriophyllum verticillatum* and *Elodea canadensis*. They may be spherical, oval, irregular and greatly elongated in shape, depending to a certain extent on the size and shape of the host cell. I have frequently found sporangia in cells of the vascular tissues which were more than 150 μ in length but only 10 μ in cross-section. They may occupy from one to as many as 13 host cells, and in such instances it is impossible to give representative measurements. Figure 20 shows a single sporangium which has spread into nine adjacent cells. Numerous sporangia of this type have been found.

Below is shown, from a count of one hundred, the number of host cells occupied by a single sporangium.

No. of Host Cells Occupied	1	2	3	4	5	6	7	8	9	10	11	12	13
No. of Sporangia Counted	26	6	16	10	10	6	5	4	4	4	4	3	2
Percentage	26%	6%	16%	10%	10%	6%	5%	4%	4%	4%	4%	3%	2%

This table shows that the majority of sporangia are confined to a single host cell and that the extremely large and irregular ones are comparatively rare.

At maturity these sporangia are filled with round and oval golden-brown refractive bodies suspended in a hyaline ground substance which appears optically homogeneous, as has been described above for the other sporangia. A single large sporangium, as is shown in figures 20 and 21, Plate XLIV, may contain as many as a thousand such globules or bodies. They thus make sharply contrasted brown flecks or spots in the more or less colorless and decaying host tissues. For this reason the sporangia may be detected with great readiness even under the 16 mm. objective magnification.

In the process of cleavage each zoöspore is formed around one of these brown globules, so that the number of zoöspores corresponds to the number of globules in the sporangium. The number of swarmspores is thus unusually great. The hyaline ground substance in which these globules lie is so homogeneous that it is impossible to know whether or not cleavage has occurred. Consequently, at maturity the sporangia look like irregular turgid vesicles filled with golden-brown spheres. Hence, it is not evident until the zoöspores begin to escape (fig. 24, Pl. XLIV) that each globule lies in a definite segment of hyaline protoplasm.

I am still uncertain as to the means and manner by which these large sporangia often come to occupy several host cells. Whether they dissolve the walls or merely burst through by pressure as they grow is not clear. Figure 20 suggests that they first make a small opening which is then apparently enlarged by growth of the sporangia. The isthmuses between the various "arms" of the sporangia in figures 21, 22, and 25 are rather narrow, but it is not uncommon to find as much as half of the wall between two adjacent cells broken through. This apparently occurs in the young stages of sporangial development, because I have often found immature sporangia (fig. 12, Pl. XLII), occupying more than one cell. I have not, however, followed the successive developmental stages of a single sporangium, and my observation rests only on isolated unrelated cases.

These irregular and large sporangia seem to be more abundant in the winter months and mature rather slowly. A large number of mature ones

have been kept under careful observation for a long time, but so far I have seen only six "germinate" or emit swarmspores. These observations might perhaps lead one to regard them as the so-called resting spores or sporangia, but in other respects they are quite unlike the resting spores described and figured by various authors for *Cladochytrium*, *Physoderma*, and *Urophlyctis*. The wall is hyaline and does not have the characteristic brown color of the resting spores. It is, furthermore, single and rather thin so that the sporangia may be easily crushed under the cover glass. Also in germination no specialized cap or area is differentiated for the escape of the zoöspores as in resting spores of *Ph. Alismatis* and *Ph. Zeae Maydis*. For these reasons I shall for the time being regard these irregular structures as perhaps late zoösporangia which are only slightly different from the ordinary type.

Structure, Behavior, and Germination of the Zoöspores

After lying quiescent at the mouth of the sporangial neck for a few moments the zoöspores become active. At first only slight movements in the cytoplasm occur; then the swarmspores become amoeboid in movement and shape, and finally separate. Once they have become active, from 2 to 24 minutes, depending on the number of zoöspores in a sporangium, are required for complete separation, according to my observations. In this process they may become considerably elongated and distorted in shape as if straining and pulling to extricate themselves and their cilia. This is particularly evident in figures 1 *m*, Plate XLII, and 8, Plate XLIII. As soon as they have separated they round up and dart off.

The most conspicuous structure of the zoöspore is the golden brown refractive body or globule which usually occupies the center of the cell. This structure enables one to recognize swarmspores at once even while they are in motion. The remainder of the cytoplasm may be finely granular or optically homogeneous in appearance, and in living material the nucleus is generally invisible. The swarmspores are usually spherical, 4 to 7 microns in diameter, and possess a single posteriorly attached cilium which is usually five or six times the diameter of the cell in length. In swimming the cilium is carried posteriorly and beats with such rapidity that it is practically invisible while the zoöspore is in motion. It is only when the swarmspore is coming to rest or starting off again that one can detect the ciliary action with certainty. They may dart back and forth with great rapidity, come to rest for a brief moment, and then dart off again. Their movement and behavior is very similar to that of the zoöspores of *Entophlyctis* and *Diplophlyctis* which I have previously reported (1928 *a*, and *b*). Figure 9 shows a group of zoöspores which appear to be entangled in their own cilia, and under such conditions they may become quite amoeboid in shape and motion again. A large number of zoöspores degenerate without germinating. The first sign of degeneration appears to be vacuolation. As is shown in figure 9, a vacuole emerges which grows larger and larger in

size until the cellular membrane is finally burst. It is thus not uncommon to find numerous refractive bodies with adjacent bits of cytoplasm and cilia on the surface of the host cells.

I have not so far followed all stages of the germination of the swarmspores and the penetration of the host cell. Although on frequent occasions large numbers of zoöspores were observed at rest on host cells, none of them germinated. I have, however, found occasional ones germinating in the water. Two such spores are shown in figure 10, Plate XLII. The penetration tubes are quite blunt in comparison with those of *Cl. tenue*, *Cl. polystomum*, and *Ph. Butomi* as they have been figured by Nowakowski, Zopf, and Büsgen. The swarmspores may germinate *in situ* as well as outside of the sporangium. In figures 11 and 12 are shown individuals which apparently failed to escape but have germinated none the less. In the former the penetration tubes have gone through the sporangium wall, while in the latter, one has grown through the neck and branched.

Host and Chytrid Relationship

Eriocaulon septangulare With. is an aquatic or bog inhabiting pipewort which has been reported in still waters and along inland shores from Newfoundland to Texas. It is thus found in the same general type of habitat in which other reported phanerogamic hosts, *Alisma*, *Butomus*, *Scirpus*, *Menyanthes*, *Trianea*, *Iris*, *Sparganium*, etc. occur. As has been noted before, this host has been growing in the laboratory for more than two years without suffering serious injury from the presence of the chytrid. The leaves die slowly, and since new ones are continually being formed the plants retain their normal appearance fairly well. To a certain degree the same conditions obtain in nature. Plants growing in more or less isolated and still regions of the pond in which the original collection was made show similar symptoms, leaves with brown and colorless tips as well as completely dead leaves in whose cells are numerous zoösporangia, rhizoids, and turbinate cells of *Cladochytrium*.

Although I have found the fungus in tips of leaves which have begun to die and traced its approximation to the healthy cells I am still uncertain as to its parasitic nature. In healthy living cells the chloroplasts and other cell contents obscure the view to such an extent that it is impossible to determine the presence of the delicate, colorless rhizo-mycelium and turbinate organs with certainty. Attempts to infect epidermal and mesophyll fragments of healthy leaves in hanging drop cultures have thus far failed. The organism may thus be only a weak parasite which enters the host cells after they have begun to die. In this respect it would be similar to *Cl. tenue* and *Now. elegans* which Nowakowski described as growing in the slime around *Chaetophora* filaments and on the surface of aquatic *Iris* leaves. The majority of reported so-called species of *Cladochytrium* and *Physoderma*, however, are markedly parasitic and attain considerable

virulence (*Ph. Alismatis*, Clinton 1902; *Ph. Butomi*, Busgen 1887; *Cl. Myriophilli*, Tostrup 1905; Ferinansen and Winge 1909; Waldie 1926).

The contents of the host cells in figure 1 show various degrees of disintegration. In many of the cells on the right of the figure the chloroplasts still retain their normal shape, but are characteristically yellow-brown in color. In other cells they are completely degenerated and clumped together in large dark brown masses. In addition numerous starch grains are present. The combination of the degeneration products together with numerous diatoms on the surface thus give the leaves their brown appearance. What the exact relation between this degeneration and the chytrid is remains to be worked out. I have yet to find the fungus in healthy green leaves and to trace its effect on the host.

Thus far I have found no evidence of hypertrophy in *Eriocaulon septangulare*. As noted before irregular sporangia may spread into adjoining cells and distend them by increasing in size, but this does not constitute hypertrophy in a true sense as in the case of *Bacillus radicicola* on legumes, for instance. In such cases the host cells are stimulated to enlargement in some unknown manner by the presence of the parasite, while in *Eriocaulon* the cells are distended or enlarged slightly through physical pressure.

Host Range

In addition to *Eriocaulon septangulare* other aquatic or semiaquatic plants appear to be host to this species of *Cladochytrium*. Up to the present time I have found six plants whose cells contained forms of *Cladochytrium* which seem morphologically identical with the one described above. *Eriocaulon*, *Spirogyra*, *Elodea*, and *Cladophora* are thus added to the host list of *Cladochytrium*. With the view of comparing these forms more concretely I am presenting the size of the zoösporangia and zoöspores, number and length of sporangial necks, and septation of the turbinate organs in table 2. With respect to the zoösporangia I have included in this table only the spherical ones, since it is almost impossible to give representative measurements of the large and irregular sporangia, which have been found only in cells of *Eriocaulon*, *Elodea*, and *Myriophyllum*.

These comparative data show that all of the forms are very similar morphologically, and it is perhaps not improbable that they constitute a single species of *Cladochytrium*, which is very ubiquitous and has a wide range of host plants. However, this cannot be determined with certainty until the resting spores have been discovered and extensive cross inoculation experiments have been made. Nevertheless, it is to be noted that these additional hosts were not in a healthy condition at the time the chytrid was found in their cells. The *Elodea*, *Myriophyllum*, *Spirogyra*, and *Cladophora* species were collected in Van Cortlandt Park in September, 1930, and grown in the same aquarium, and none of them showed decided infection until numerous cells had begun to die more than a month later.

TABLE 2. *The Comparative Size and Structure of Zoösporangia, Zoöspores, and Turbinate Organs of Cladochytrium sp. in Various Host Plants*

Host Plant	Locality	Diam. of Spherical Sporangia	Zoösporangia	Diam. of Zoöspores	Zoöspores	No. and Length of Sporangial Necks	Type of Turbinate Organs
Eriocaulon septangulare	Long Island, New York	10–17 μ	Predominantly terminal, filled with golden brown globules at maturity	4.5–6 μ	Spherical hyaline except for a highly refractive golden-brown globule in the center	Predominantly 5 to 60 μ	1- to 6-celled
Eriocaulon compressum	Southern New Jersey	11–17 μ	"	5–7 μ	"	Predominantly 1, 8 to 35 μ	1- to 4-celled
Elodea canadensis	Van Cortlandt Park, N. Y. City	10–16 μ	"	4–6 μ	"	Predominantly 1, 5 to 25 μ	1- to 5-celled
Myriophyllum verticillatum	"	11–16 μ	"	4–6 μ	"	Predominantly 1, 10 to 40 μ	1- to 6-celled
Cladophora glomerata	"	10–17 μ	"	4–7 μ	"	Predominantly 1, 4 to 20 μ	1- to 3-celled
Spirogyra sp.	"	10–17 μ	"	4–7 μ	"	Predominantly 5–30 μ	Predominantly 2-celled

That death did not result entirely from the presence of *Cladochytrium* was evident from the fact that many of the dead plants were not infected.

The degree of pathogenicity of the form occurring in cells of *Spirogyra* and *Cladophora* was difficult to determine, since the initial infection stages were not observed. The appearance of the infected cell suggested at once that the organism is a virulent parasite, but it is, none the less, uncertain whether the degenerating and pathological condition was due to the presence of the chytrid, or whether the parasite had gained entrance only after such a condition had been established. Careful study of the initial infection stages and the progress of the disease must first be made before this problem can be solved.

Thus, from the standpoint of pathogenicity, as far as my observations go at the present time, these forms from various hosts appear to be very similar. Evidence at hand seems to indicate that they constitute a single ubiquitous species which spreads readily from one aquatic outdoor or greenhouse plant to another. Extensive cross-inoculation experiments with *Eriocaulon*, *Elodea*, *Myriophyllum*, *Acorus*, *Butomus*, *Iris*, *Sagittaria*, *Cladophora*, *Oedogonium*, *Chaetophora*, *Spirogyra*, *Vaucheria*, etc., as host plants are under way at present to determine the range of hosts.

At this point it is worth while to call attention to Dangeard's (1889) description of *Chytridium Elodeae* from the cortical cells of *Elodea canadensis*. The zoösporangia and zoöspores are 30 μ and 3 μ in diameter, respectively. Although he was unable to find rhizoids or a rhizo-mycelium, Dangeard, none the less, thinks it might possibly be a species of *Cladochytrium*, since the zoöspores escape from the sporangia and lie quiescent at the mouth of the neck. They then gradually begin to disentangle themselves in the same manner as I have described for *Cladochytrium*. It is therefore highly probable that Dangeard was dealing with a species of *Cladochytrium* and had overlooked the rhizo-mycelium.

Identity of the Organism

Since I have thus far failed to find the resting spores the exact relationship of this species of *Cladochytrium* is uncertain. Whether or not it is *Cl. Myrophylli* described by Rostrup (1904) and Ferdinandsen and Winge (1909) on *M. verticillatum* is uncertain. These authors failed to find the zoösporangia and thus figure only resting spores with attached "Nebenzellen," "Sammelzellen," while I have found only zoösporangia. There are certain characteristics, however, which may prove significant in differentiating the two. Whereas *Cl. Myriophylli* is parasitic and causes tumors on the stems and branches of *M. verticillatum*, our species is apparently weakly parasitic and appears to have little effect on the same host. Furthermore, Ferdinandsen and Winge found that *Cl. Myriophylli* was limited to one host, *M. verticillatum*, and would not in spite of cross-inoculation experiments go to *M. alterniflorum*, while ours appears to have a wide range of hosts.

From the standpoint of color our species is similar to *Cl. polystomum*, which Zopf (1885) figured in cells of *Trianea*. Zopf did not describe this species, but according to his drawings (figs. 5 and 6, Pl. 21) the content of the sporangia is finely granular and golden brown in color. When the zoöspores are fully formed this color is aggregated into highly refractive golden brown bodies or globules (figs. 5 and 11, Pl. 21) which occupy the center of the spore in the same manner as I have described above for *Cladochytrium* sp. On the other hand, the sporangia of *Cl. polystomum* have a great number of necks for the escape of the zoöspores, while those of *Cladochytrium* sp. have rarely more than one, as far as my observations go.

However, it is obviously questionable whether the number of sporangial necks and the color of the sporangium content are specific morphological characters. In *Entophlyctis* and *Diplophlyctis*, for instance, the number of necks is usually one, but when the host tissue is badly decayed and rich in food a great profusion of sporangial necks occurs. Similarly, refractive, golden brown bodies occur in the zoöspores of other chytrids, *Polyphagus Euglenae* (Nowakowski, 1877 *b*), *Zygochytrium aurantiacum* (Sorokine, 1876), *Amoebochytrium rhizidioides* (Zopf, 1885), etc., for instance, which we know are unrelated, while the content of the resting sporangia of *Physoderma Heleocharidis*, according to Fuckel (1869), is also of the same color.

While this species seems closely related to *Cl. tenue* and *Cl. polystomum* in general appearance and structure it has several distinctive specific characteristics. For the sake of clarity and convenience in further studies and in order to emphasize the replication of the thallus which seems to be one of the outstanding characteristics of the family Cladochytriaceae I have chosen the specific name *replicatum* for our species. Further studies may prove it to be synonymous with *Cl. tenue* or *Cl. polystomum*, and in that case either of these specific names should take precedence.

Cladochytrium replicatum n. sp.

Thallus intramatrical, consisting of a fine effervescent, tenuous branched "rhizo-mycelium" with one to several celled turbinate organs which is more or less regularly replicated as it spreads through the host tissue. Sporangia spherical, oval or irregular, depending often on the size and shape of the host cell; spherical sporangia 8–18 μ in diameter, proliferating, usually with one neck for the discharge of zoöspores. Zoöspores spherical with a bright golden brown globule and one posteriorly attached cilium; 4–7 μ in diameter. Number of zoöspores per sporangium variable, escaping through the sporangial neck and lying quiescent in a mass for a few seconds before swimming away. Resting spores unknown. Parasitic or weakly so on *Eriocaulon septangulare*, *E. compressum*, *Elodea canadensis*, *Myriophyllum verticillatum*, *Cladophora glomerata*, and *Spirogyra* sp. Long Island, N. Y., and Van Cortlandt Park, New York City.

General Considerations as to the Family Cladochytriaceae

The great confusion that exists in the literature as to the classification and phylogeny of the Chytridiales is perhaps nowhere better illustrated

than in the treatment accorded the genera of the Cladochytriaceae. Most of this confusion has arisen, it seems to me, by the establishment of innumerable new species on only fragmentary knowledge of their life histories. This has necessitated an almost constant shifting of generic and specific distinctions with each bit of new evidence that has been brought to light, and, as a consequence, many species which have been repeatedly described bear a long list of synonyms. However, this confusion will continue to exist, and there seems little hope of a better knowledge of the phylogeny and relationships of the group until all of the known species have been intensively reinvestigated. In attempting to classify the species which I have studied I have become acutely aware of the difficulties as they exist at the present time, and am thus presenting in a somewhat historical manner the conflicting opinions and data as they have been given. I do not, however, at this stage attempt to say what constitutes legitimate species or to offer an alternative system of classification.

While, as far as our present knowledge goes, this family seems fairly distinct from the Rhizidiaceae by the replication of its vegetative thallus, terminal or intercalary sporangia and resting spores, and the presence of turbinate enlargements on an effervescent rhizo-mycelium, its limits are not so sharply defined in the literature. In 1889 Schröter made the Cladochytriae a subfamily of the Rhizidiaceae with two genera, *Cladochytrium* and *Physoderma*. Later (1892) Fischer established the family Cladochytriaceae as synonymous with Hyphochytriaceae and included four genera, *Physoderma*, *Cladochytrium*, *Amoebochytrium*, and *Nowakowskiella*. Since that time by von Minden (1911–1915), Gäumann (1926), Gwynne-Vaughan and Barnes (1927), and Fitzpatrick (1930), the family has been extended to include Schröter's genus *Urophlyctis* of the family Oochytriaceae. An additional new genus, *Rhizidiocystis*, has been (1929) recently established by Sideris, which he believes belongs to this family. Fitzpatrick, whose book (1930) on the Phycomycetes is the most recent classification to date, follows Fischer in including the genus *Catenaria*, which Dangeard (1885) regarded as a member of the Ancylistales. Furthermore, he excludes the Hypochytriaceae and makes the Cladochytriaceae the climax family of the Chytridiales.

It is, however, particularly with the genera *Cladochytrium*, *Physoderma*, and *Urophlyctis* that we are at present concerned. *Physoderma* was established by Wallroth in 1833 as a genus of Sporomycetes with three species, *Ph. maculare*, *Ph. gibbosum*, and *Ph. pulposum*, to include a group of fungi which he found parasitizing and causing discoloration of the leaves of phanerogamic plants. He characterizes the genus as follows: "Sporidia simplicia sphaerica insignia ex massa sporacea opaca, parenchyma herbarum vegetarum penetrantia grumulosque convexos s. gibbosas epidermide relatos informantia."

This description of *Physoderma* is almost identical with that for

Protomyces, a new genus which Unger (1833) had described during the same year in his book on "Die Exantheme der Pflanzen." Wallroth, according to De Bary (1864), included *Prot. macrosporus* in his genus as *Ph. gibbosum;* and thus at the outset the names *Physoderma* and *Protomyces* became involved. This led De Bary (1853–1864) and Fuckel (1860–1869) to use *Protomyces* for a number of species which have since proven to be chytrids. Bonorden (1851) regarded *Protomyces* as a genus closely related to the smuts and used the name of *Physoderma* for a group of the rusts. In 1864 De Bary reëxamined Wallroth's original material and made a careful comparative study of the life histories of other species of *Protomyces* and *Physoderma*, and came to the conclusion that although the habitat and habit of growth as well as the symptoms produced on the host plants seemed very similar, the two genera, none the less, were by and large distinct.

Protomyces continued to be used, however, by Fuckel (1866–1869), who included most of the known species of *Physoderma* under this name; but in 1874 De Bary separated what he considered the four legitimate chytrid species from *Protomyces*, *Entyloma*, and *Malanotaenium* of the Ustilaginaceae and placed them in the genus *Physoderma*. At this time, however, he was still uncertain as to its family relationship, and thus the genus remained in an undetermined position until after Nowakowski (1877) had founded *Cladochytrium* as a genus of the Chytridiaceae. *Cladochytrium* at first included only two species, *Cl. elegans* and *Cl. tenue*, which have a delicate, branched mycelium-like rhizoidal system and bear terminal or intercalary zoösporangia which shed their spores either through a long neck or a lid-like opening. In 1884 De Bary recognized and extended this genus to include some additional species and pointed out the close resemblance of these species to his own *Protomyces menyanthes*, Wallroth's *Ph. maculare*, Fuckel's *Ph. Heleocharidis*, and Schröter's (1882 *a*) *Ph. Butomi* and *Ph. vagans*. Previously Schröter (1882 *b*, p. 221) had called attention to the similarity between the formation of the resting spores of *Physoderma* and those of certain chytrids; and this statement together with that of De Bary mentioned above is perhaps the first intimation in the literature, as far as I am aware, of the relationship of *Physoderma* to the Chytridiales. In 1883, however, Cornu included *Ph. maculare* in the genus *Melanotaenium* of the Ustilaginales, but as noted before, in 1889 Schröter placed the two genera under the subfamily Cladochytriae of the Rhizidiaceae. They were finally incorporated in a distinct family, Hyphochytriaceae or Cladochytriaceae, by Fischer (1892).

Schröter (1889–1897) separated the two genera principally on the fact that resting spores had not been found in *Cladochytrium* while ephemeral zoösporangia were unknown in *Physoderma*. In the same year (1889) he established the genus *Urophlyctis* under the family Zygochytriaceae to include Wallroth's *Ph. pulposa* and *Uro. majus*, whose resting spores he

believed were formed by the conjugation of two similar cells, the contents of one of which flowed into the other, the empty cell thus remaining being attached as an antheridium to the developing resting spore. Thus *Physoderma* became involved with *Urophlyctis* in addition to *Protomyces*, *Melanotaenium*, and *Cladochytrium*.

Fischer denied the presence of sexuality and made *Urophlyctis* together with *Cladosporangium* and *Physoderma* subgenera of *Cladochytrium*. He based his classification like Schröter chiefly on the presence or absence of zoösporangia and their position relative to the host tissue. Without any consideration of Fischer's classification, Schröter (1897) in Engler and Prantl's Natürlichen Pflanzenfamilien retains *Physoderma* and *Cladochytrium* in the family Cladochytriaceae and refers *Urophlyctis* to his new family Oöchytriaceae, which thus takes the place of his former Zygochytriaceae. Schröter's claim of sexuality in *Urophlyctis* was strongly supported by Magnus (1897–1901–1902) but subsequent cytological studies (Jones and Drechsler, 1921, and Bartlett, 1926) have denied the presence of sexual fusion in the formation of the resting spores.

It is obvious from this brief historical resume that the original distinctions between *Physoderma* and *Urophlyctis* have broken down and are no longer tenable. Seeking a new basis for separation von Minden (1911–1915), and after him Fitzpatrick, include in *Physoderma* those species which cause only discoloration or slight hypertrophy of the host tissues, while *Urophlyctis* is restricted to those which produce pronounced hypertrophy or deformation. While such a distinction may serve in a general way to separate the two genera it rests, none the less, not on morphological differences between the organisms themselves but on the effect they produce on the host plant. Furthermore, it is very difficult to draw a sharp line between discoloration or slight hypertrophy and pronounced hypertrophy or deformation, because in the whole range of *Physoderma* and *Urophlyctis* species may be found various degrees of discoloration, hypertrophy, and deformation.

Magnus (1901), Bartlett (1926), Gäumann (1929), and Gäumann and Dodge (1928), on the other hand, do not attach particular significance to the presence or absence of the zoösporangium as a basis of classification and maintain that there is a marked morphological difference in the shape of the resting spores, *Physoderma* having spherical or ellipsoidal spores while those of *Urophlyctis* are flattened on one side or hemispherical. While this view is supported in a general way by the drawings of resting spores in the literature, it is not safe to make too sharp and fast lines of separation on this basis, because the shape of the spores doubtless varies greatly in different species of the same genus. Accurate measurements, drawings, and careful comparison of the resting spores of all known species must first be made before any sharp delimitation can be drawn.

As to the genus *Cladochytrium* the lines of separation are no less distinct than in the two previous genera. The tendency of Schröter (1889–1897),

Magnus (1901), Minden (1911–1915), Fitzpatrick (1930), and others to limit *Cladochytrium* to *Physoderma*- and *Urophlyctis*-like species in which no resting spores have been found has by no means received universal support (De Bary 1884; Büsgen 1887; Fischer 1892; Vuillemin 1894; Lagerheim 1898; Ludi 1901; Clinton 1902; Migula 1910; Gwynne-Vaughan and Barnes 1927; etc.). Such a basis of classification limits the genus to the two original species of Nowakowski, *Cl. tenue* and *Cl. elegans*. In 1897, however, Schröter made *Cl. elegans* the type species of his new genus *Nowakowskiella*, leaving, in Fitzpatrick's opinion, only one legitimate species, *Cl. tenue*. We have thus but to find the resting spores of this species, and the genus *Cladochytrium* will exist only in name!

None the less, there seems to be a distinction, as far as our present knowledge goes, between the manner in which the zoösporangia of *Now. elegans* and *Cl. tenue* and those of some of the so-called *Physoderma* and *Urophlyctis* species are formed. In the former the zoösporangia develop intercalary or terminal on a rhizo-mycelium which has developed from a germinated zoöspore; while in *Physoderma maculare*, *Ph. Butomi*, and *Urophlyctis pulposum* they arise directly from the germinating zoöspore and develop near or on the surface of the host leaf. How far this distinction of Fischer's can be extended remains to be seen as our knowledge of the life histories of these different species increases.

With the view of presenting more concretely the conflicting opinions as to which species should be included in *Cladochytrium* I have listed in table 3 some of the generic and specific names such species have been given from time to time by different authors. By listing them all as *Cladochytrium* species at the top of the table, I do not intend to favor such a grouping, but merely to indicate that at one time or another they have been placed in this genus. A similar table of the *Urophlyctis* species will doubtless show the same conflicts of opinion.

In this table it will be seen that Wallroth's original *Ph. maculare* has been placed in no less than four different genera, and Fischer's *Cl. kriegerianum*, in six, including both the smuts and rusts. Comparatively few species retain their original generic and specific names, and these chiefly because they have been found but once or twice. This table emphasizes even further the breakdown of generic distinction between *Cladochytrium*, *Physoderma*, and *Urophlyctis* and the need of revision in classification. At our present state of knowledge of these three genera much is to be said in favor of Fischer's attempt to group them under a single large genus, and we may perhaps eventually come to such a viewpoint.

I shall now discuss very briefly the other genera which have been included in the Cladochytriaceae. The genus *Nowakowskiella* was founded by Schröter in 1897 to include Nowakowski's *Cl. elegans* which grows more or less saprophytically in the slime of *Chaetophora elegans* and whose sporangia open by a lid rather than an extended neck as in *Cl. tenue*.

TABLE 3. *Summary of Cladochytrium, Physoderma, and Urophlyctis Species*

	Cl. alismatis	*Cl. menyanthis*	*Cl. tenue*	*Cl. elegans*	*Cl. butomi*	*Cl. pulposum*	*Cl. iridis*
Wallroth (1833)	*Ph. maculare*					*Ph. pulposum*	
DeBary (1853)		*Proto. menyanthis*					
DeBary (1864)	*Ph. maculare*	*Proto. menyanthis*				*Ph. pulposum*	
DeBary (1874)	*Ph. maculare*	*Ph. menyanthis*				*Ph. pulposum*	
DeBary (1884)	*Ph. maculare*	*Cl. menyanthis*			*Ph. butomi*		*Cl. iridis*
Fuckel (1860)		*Ph. menyanthis*					
Fuckel (1869)	*Proto. macularis*	*Proto. menyanthis*					
Schröter (1870)							
Schröter (1882)							
Schröter (1889)	*Ph. maculare*	*Ph. menyanthis*	*Cl. tenue*	*Cl. elegans*		*Urophlyctis pulposa*	
Schröter (1897)	*Ph. maculare*	*Ph. menyanthis*	*Cl. tenue*	*Now. elegans*	*Urophlyctis butomi*	*Urophlyctis pulposa*	
Nowakowski (1877)			*Cl. tenue*	*Cl. elegans*			
Spegazzini (1881)							
Spegazzini (1902)							
Spegazzini (1903)							
Spegazzini (1909)						*Urophlyctis pulposa*	
Cornu (1883)	*Melanotaenium alismatis*						
Zopf (1885)							
Büsgen (1887)	*Cl. alismatis*	*Cl. menyanthis*			*Cl. butomi*		*Cl. iridis*
Berlese & De-Toni (1888)	*Ph. maculare*	*Ph. menyanthis*	*Cl. tenue*	*Cl. elegans*		*Urophlyctis pulposa*	
Magnus (1888)							
Magnus (1897)					*Ph. butomi*	*Urophlyctis pulposa*	
Magnus (1901)						*Urophlyctis pulposa*	
Magnus (1902)							
Fischer (1892)	*Cl. alismatis*	*Cl. menyanthis*	*Cl. tenue*	*Cl. elegans*	*Cl. butomi*	*Cl. pulposum*	*Cl. iridis*
Prunet (1894)							
Patouillard & Lagerheim (1895)							
Patouillard (1897)							
Wildeman (1897)							
Vuillemin (1896)							
Vuillemin (1897)							
Lüdi (1901)		*Cl. menyanthis*			*Cl. butomi*		
Constantineanu (1901)	*Ph. maculare*		*Cl. tenue*				
Clinton (1902)	*Cl. alismatis*	*Cl. menyanthis*			*Cl. butomi*		
H. & P. Sydow (1903)						*Urophlyctis pulposa*	
Rostrup (1904)							
Ferdinandsen & Winge (1909)							
Petersen (1909, 1910)			*Cl. tenue*				
Migula (1910)	*Ph. maculare*	*Ph. menyanthis*	*Cl. tenue*		*Urophlyctis butomi*	*Urophlyctis pulposa*	*Ph. iridis*
Maire & Tison (1910)							
Minden (1911–1915)	*Ph. maculare*	*Ph. menyanthis*	*Cl. tenue*		*Ph. butomi*	*Urophlyctis pulposa*	*Ph. iridis*
Tisdale (1919)							
Jones & Drechsler (1920)	*Ph. maculare*	*Ph. menyanthis*			*Ph. butomi*	*Urophlyctis pulposa*	*Ph. iridis*
Tanaka (1922)							
Gäumann (1926)	*Ph. maculare*		*Cl. tenue*			*Urophlyctis pulposa*	
Gwynne-Vaughan & Barnes (1927)	*Cl. alismatis*						
Gäumann & Dodge (1928)	*Ph. maculare*		*Cl. tenue*				
Fitzpatrick (1930)	*Ph. maculare*	*Ph. menyanthis*	*Cl. tenue*	*Now. elegans*		*Urophylctis pulposa*	

TABLE 3—*Continued*

	Cl. graminis	*Cl. sparganii ramosi*	*Cl. heleocharidis*	*Cl. menthae*	*Cl. myriophylli*	*Cl. flammulae*	*Cl. polystomum*
Wallroth (1833)							
DeBary (1853)							
DeBary (1864)							
DeBary (1874)			*Ph. heleocharidis*				
DeBary (1884)			*Ph. heleocharidis*				
Fuckel (1860)			*Proto. heleocharidis*				
Fuckel (1869)			*Proto. heleocharidis*				
Schröter (1870)				*Ustilago menthae*			
Schröter (1882)							
Schröter (1889)			*Ph. heleocharidis*	*Ph. menthae*			
Schröter (1897)			*Ph. heleocharidis*				
Nowakowski (1877)							*Cl. polystomum*
Spegazzini (1881)							
Spegazzini (1902)							
Spegazzini (1903)							
Spegazzini (1903)							
Cornu (1883)							
Zopf (1885)							
Büsgen (1887)							
Berlese & De-Toni (1888)	*Cl. graminis*	*Cl. sparganii ramosi*	*Cl. heleocharidis*				*Cl. polystomum*
Magnus (1888)			*Ph. heleocharidis*	*Ph. menthae*			*Cl. polystomum*
Magnus (1897)							
Magnus (1901)							
Magnus (1902)							
Fischer (1892)							
Prunet (1894)	*Cl. graminis*	*Cl. sparganii ramosi*	*Cl. heleocharidis*	*Cl. menthae*			
Patouillard & Lagerheim (1895)						*Cl. flammulae*	*Cl. polystomum*
Patouillard (1897)							
Wildeman (1897)							
Vuillemin (1896)							
Vuillemin (1897)							
Lüdi (1901)							
Constantineanu (1901)							
Clinton (1902)							*Cl. polystomum*
H. & P. Sydow (1903)							
Rostrup (1904)							
Ferdinandsen & Winge (1909)					*Cl. myriophylli*		
Petersen (1909, 1910)					*Cl. myriophylli*		
Miguła (1910)	*Ph. graminis*	*Ph. sparganii ramosi*	*Ph. heleocharidis*	*Ph. menthae*	*Cl. myriophylli*		*Cl. polystomum*
Maire & Tison (1910)							
Minden (1911–1915)	*Ph. graminis*	*Ph. sparganii ramosi*	*Ph. heleocharidis*	*Ph. menthae*			
Tisdale (1919)						*Ph. flammulae*	*Cl. polystomum*
Jones & Drechsler (1920)	*Ph. graminis*	*Ph. sparganii ramosi*	*Ph. heleocharidis*	*Ph. menthae*		*Ph. flammulae*	
Tanaka (1922)							
Gäumann (1926)							
Gwynne-Vaughan & Barnes (1927)							
Gäumann & Dodge (1928)							
Fitzpatrick (1930)			*Ph. heleocharidis*				*Cl. polystomum*

TABLE 3—*Continued*

	Cl. kriegeranium	*Cl. vagans*	*Cl. majus*	*Cl. Gerhardti*	*Cl. maydis*	*Cl. speciosum*	*Cl. alfalfae*
Wallroth (1833)							
DeBary (1853)							
DeBary (1864)							
DeBary (1874)							
DeBary (1884)		*Ph. vagans*					
Fuckel (1860)							
Fuckel (1869)							
Schröter (1870)							
Schröter (1882)							
Schröter (1889)		*Ph. vagans*	*Urophlyctis majus*	*Ph. Gerhardti*		*Ph. speciosum*	
Schröter (1897)		*Ph. vagans*	*Uroph. major*	*Ph. Gerhardti*			
Nowakowski (1877)							
Spegazzini (1881)	*Uromyces hemisphericus*						
Spegazzini (1902)	*Entyloma hemisphericum*						
Spegazzini (1903)	*Oedomyces hemisphericus*						
Spegazzini (1909)	*Urophlyctis hemisphaeria*						*Urophlyc. alfalfae*
Cornu (1883)							
Zopf (1885)							
Büsgen (1887)							
Berlese & De-Toni (1888)		*Ph. vagans*	*Uroph. major*	*Ph. Gerhardti*		*Ph. speciosum*	
Magnus (1888)	*Urophlyctis kriegeriana*						
Magnus (1897)	*Urophlyctis kriegeriana*						
Magnus (1901)	*Urophlyctis kriegeriana*						
Magnus (1902)							*Urophlyc. alfalfae*
Fischer (1892)	*Cl. kriegerianum*	*Cl. vagans*	*Cl. majus*	*Cl. Gerhardti*		*Cl. speciosum*	
Prunet (1894)							
Patouillard & Lagerheim (1895)							*Cl. alfalfae*
Patouillard (1897)	*Cl. kriegerianum*						
Wildeman (1897)							
Vuillemin (1896)							
Vuillemin (1897)							
Lüdi (1901)							
Constantineanu (1901)							
Clinton (1902)							
H. & P. Sydow (1903)	*Urophlyctis hemisphaerica*						*Urophy. alfalfae*
Rostrup (1904)							
Ferdinandsen & Winge (1909)							
Petersen (1909, 1910)	*Urophlyctis kriegeriana*	*Ph. vagans*	*Uroph. major*	*Ph. Gerhardti*		*Ph. speciosum*	*Urophy. alfalfae*
Migula (1910)							
Maire & Tison (1910)	*Urophlyctis hemasphaerica*						
Minden (1911–1915)	*Urophlyctis kriegeriana*		*Uroph. majcr*	*Ph. Gerhardti*		*Ph. speciosum*	*Urophy. alfalfae*
Tisdale (1919)					*Ph. Zeae maydis*		
Jones & Drechsler (1920)	*Ph. kriegeriana*	*Ph. vagans*		*Ph. Gerhardti*	*Ph. Zeae maydis*	*Ph. speciosum*	*Uroph. alfalfae*
Tanaka (1922)					*Cl. maydis*		
Gäumann (1926)					*Ph. Zeae maydis*		*Urophlyc. alfalfae*
Gwynne-Vaughan & Barnes (1927)							*Urophlyc. alfalfae*
Gäumann & Dodge (1928)							
Fitzpatrick (1930)	*Urophylcis kriegeriana*	*Ph. vagans*	*Uroph. major*		*Ph. Zeae maydis*		

TABLE 3—*Continued*

	Cl. viticolum	*Cl. brevierei*	*Cl. hippuridis*	*Cl. cornutum*	*Cl. irregulare*	*Cl. asphodeli*	*Cl. urgineae*
Wallroth (1833)							
DeBary (1853)							
DeBary (1864)							
DeBary (1874)							
DeBary (1884)							
Fuckel (1860)							
Fuckel (1869)							
Schröter (1870)							
Schröter (1882)							
Schröter (1889)							
Schröter (1897)							
Nowakowski (1877)							
Spegazzini (1881)							
Spegazzini (1902)							
Spegazzini (1903)							
Spegazzini (1909)							
Cornu (1883)							
Zopf (1885)							
Büsgen (1887)							
Berlese & De-Toni (1888)							
Magnus (1888)							
Magnus (1897)							
Magnus (1901)							
Magnus (1902)							
Fischer (1892)							
Prunet (1894)	*Cl. viticolum*						
Patouillard & Lagerheim (1895)							
Patouillard (1897)							
Wildeman (1897)							
Vuillemin (1896)				*Cl. cornutum*	*Cl. irregulare*	*Cl. asphodeli*	*Cl. urgineae*
Vuillemin (1897)							
Lüdi (1901)	*Cl. viticolum*						
Constantineanu (1901)							
Clinton (1902)							
H. & P. Sydow (1903)							
Rostrup (1904)							
Ferdinandsen & Winge (1909)							
Petersen (1909, 1910)	*Cl. viticolum*	*Cl. brevierei*	*Cl. hippuridis*	*Cl. cornutum*	*Cl. irregulare*		
Migula (1910)							
Maire & Tison (1910)							
Minden (1911–1915)							
Tisdale (1919)			*Cl. hippuridis*				
Jones & Drechsler (1920)							
Tanaka (1922)			*Ph. hippuridis*				
Gäumann (1926)							
Gwynne-Vaughan & Barnes (1927)							
Gäumann & Dodge (1928)							
Fitzpatrick (1930)				*Cl. cornutum?*	*Cl. irregulare?*		

According to Nowakowski's drawings the rhizo-mycelium is also more irregular in diameter, particularly in the region where branches arise, and the turbinate enlargements less uniform and definite in size and shape. In none of his figures, however, are they shown as multicellular or even delimited from the remainder of the rhizo-mycelium by septa. In Miss Matthews' (1928) drawings, however (figs. 1 and 2, Pl. 34), they are figured as more definitely spindle-shaped but still continuous with the rest of the thallus.

Another species, *Now. endogena*, was described by Constantineanu in 1901 which is similar to Nowakowski's *Cl. elegans* in that the sporangia proliferate and open by an operculum, but these have, none the less, a tube or neck for the exit of the zoöspores. Constantineanu considers his species intermediate between *Cladochytrium* and *Nowakowskiella* and seriously questions the generic rank of the latter. On the basis of whether a sporangium opens by an operculum or possesses a neck for the emission of the zoöspores, his skepticism, it seems to me, is well justified. The length of the sporangial neck as well as the manner in which the sporangium opens, doubtless varies under different conditions. In *Diplophlyctis* (Karling, 1930), for instance, the neck may be extremely long or so reduced as to be indistinguishable from the sporangium itself. In the latter cases the sporangium thus opens in a definite region at the apex which under the circumstances might be regarded as a lid or an operculum.

E. J. Butler (1907), on the other hand, has given a sounder basis to the genus by the discovery and description of the process of resting spore formation in *Now. ramosa* which he found growing on decaying wheat stems in India. In this species the rhizo-mycelium is more extensively developed than in any of the Cladochytriaceae hitherto described. Its filaments are thick-walled and highly branched, and anastomose freely, forming numerous irregular junctions. According to Butler's description and figures it is strikingly similar to the mycelium of the higher fungi. The turbinate enlargements are no more uniform and definite than in *Now. elegans*, and their existence as distinct cellular organs is somewhat doubtful, except perhaps in relation to the resting spores where they have a marked dictyosporous-like appearance, which is due to an extensive proliferation and division of the ends of the vegetative filaments.

The resting spores begin as enlargements of the terminal or marginal cells of the proliferated masses. These cells increase in size, become spherical, and finally develop thick walls. In cases of extended proliferation a group of resting spores are developed which seem joined together by a mass of parenchyma-like cells. Butler has not observed germination of the resting spores, and it is still unknown whether or not zoöspores are formed.

I have mentioned several times above the occurrence of turbinate organs or "Sammelzellen" in the genus *Nowakowskiella*, but it is obvious

from the descriptions and figures given by Nowakowski, Constantineanu, Butler, and Matthews that if they exist at all they are not as definite as in *Cladochytrium*, *Physoderma*, and *Urophlyctis*. Butler, however, regards the "hyphal" proliferation and division in relation to the formation of the resting spores as an extreme development of the Sammelzellen of the Cladochytriaceae. The same doubtful conditions obtain by and large, as we shall see, in *Catenaria* and *Rhizidiocystis* and to a certain extent in *Amoebochytrium*, and whether or not these irregularities in the diameter of the rhizo-mycelium are to be regarded as Sammelzellen or turbinate organs depends, of course, on our definition of such structures. In a later paper I shall discuss these structures more in detail.

The genus *Amoebochytrium* which at present is confined to a single species, *A. rhizidioides* (Zopf, 1884), differs from all other genera in that the zoöspores lack cilia and are markedly amoeboid. Like *Now. elegans* it occurs in the slime of *Chaetophora* or may become parasitic to a certain extent in cells of this alga. The zoösporangia develop as enlargements on the rhizo-mycelium (Zopf, fig. 4, Pl. 6) which are then cut off by transverse septa. One end of the delimited enlargement increases in diameter, while the other enlarges but little and becomes the sporangial neck. Thus, in this species the neck is but a portion of the rhizo-mycelium, according to Zopf, and the amoeboid zoöspores must break through the transverse wall to escape. The turbinate organs, if they exist at all, are not very highly developed. At the base of the zoösporangium (Zopf, figs. 6, 7, 9, and 11, Pl. 6) is a swollen cell which resembles the apophysis of *Diplophlyctis* of the Rhizidiaceae, while at various intervals on the rhizo-mycelium occur other enlargements. In view of what is known concerning the turbinate organs in relation to the zoösporangia and resting spores in *Cladochytrium*, *Physoderma*, and *Urophlyctis* it seems that the apophysis-like structure figured by Zopf may be one cell of a turbinate organ whose second cell then enlarged into a sporangium. Zopf does not, however, give a detailed account of sporangium development, and until the species has been further studied it is premature to draw any conclusions.

Zopf regarded *Amoebochytrium rhizidioides* as an intermediate form between the Rhizidiaceae and the Cladochytriaceae. It was first definitely incorporated in the Cladochytriaceae by Fischer and has been retained in this family by Schröter (1897), Migula, von Minden, Gäumann, Gwynne-Vaughan and Barnes, Gäumann and Dodge, and Fitzpatrick. In its general habit of growth and development it seems to be fairly primitive, but it is very questionable whether it is the ancestor of the seemingly more highly developed species of Cladochytriaceae. It is to be noted, however, that in general in its habitat and pathogenicity it is very similar to *Nowakowskiella*, *Cl. tenue*, and *Cladochytrium replicatum* which I have described above.

We come thus to the three remaining genera. *Catenaria* was founded by Sorokine in 1876, 1883, and 1889 for a chytrid which he found attacking

eelworms and to which he gave the name *C. Anguillulae*. It seems, however, that this same species apparently had been described and figured two years previously by Villot (1874), who took it to be a freshwater alga parasitic in several species of hairworms of the genus *Gordius*. It is furthermore highly probable that *Chytridium zootocum* which Braun (1856) described in dead Anguillulae may be *Catenaria Anguillulae*. His description of the sporangia fits quite well, but he failed to observe rhizoids. This may, however, have been due to oversight, since the delicate rhizoids which extend from the isthmuses between the sporangia and from the sporangia themselves are often difficult to see in the body of the worm.

Since the time of Sorokine, Dangeard (1885, 1886), Constantineanu (1901), J. B. Butler and Buckley (1927), E. J. Butler (1928), and Buckley and Clapham (1929) have described it from various sources, so that at present its life cycle is fairly well known. The evidence as to whether or not Serbinov's (1907) *C. pygmaea* from cells of *Mougeotia* is a distinct species has been well reviewed by von Minden (1911–1915) and E. J. Butler (1928), and I need not discuss this evidence further at the present time.

From the descriptions and figures in the literature the thallus of *Catenaria* consists of a dichotomously branched rhizo-mycelium which quite early becomes irregular and swollen at frequent intervals, so that in comparison the fine end branches remain to all appearances as delicate thin-walled rhizoids attached to the swollen regions and isthmuses between them. As the enlarged portions increase in size they are usually cut off from the remainder of the thallus by septa and finally become large, round and irregular sporangia. For this reason at maturity the thallus appears septate and composed of a series of large more or less closely adjoining zoösporangia with numerous fine attached rhizoids. This is particularly true in infected infusorial cysts and liver-fluke eggs where the thallus usually becomes compactly crowded together. In such cases the sporangia may be so close together that it becomes almost impossible to distinguish the isthmuses and so-called rhizoids (Butler, E. J., 1928, fig. 18). This fact led Dangeard to seriously question whether Braun's (1856) *Ch. endogenum*, Nowakowski's *Ch. gregarium*, Schröter's (1897) *Olpidium gregarium*, and Sorokine's (1876) *Achlyogeton rostratum* might not be reduced specimens of *C. Anguillulae* in which the remainder of the thallus had been overlooked.

In none of the descriptions or figures of *Catenaria* so far given are definite turbinate organs or Sammelzellen indicated. However, Dangeard (1885) has figured enlargements on the thallus, particularly near the region of entry and infection, which are somewhat similar to those on the rhizo-mycelium of *Now. elegans* (Nowakowski, fig. 15, Pl. 6). Unless we regard these enlargements as such or postulate that all turbinate organs or cells are transformed into zoösporangia they apparently do not exist in this genus as far as our knowledge goes at the present time.

Catenaria was first placed in the family Hyphochytriaceae or Clado-

chytriaceae by Fischer. Schröter likewise placed it in the Hyphochytriaceae, but it is to be noted that his family is not synonymous with Fischer's Hyphochytriaceae. Fischer's classification has been supported by von Minden, E. J. Butler (1928), J. B. Butler and Buckley, and Fitzpatrick. Migula follows Schröter's grouping and places it next to *Hyphochytrium*, *Polyrrhina*, and *Tetrachytrium*. Dangeard, on the other hand, includes *Catenaria* with the Ancylistales. There is much to be said for Dangeard's view from the standpoint of the appearance of the thallus. The thallus of the Ancylistales is in general composed of thick irregular segments which either become transformed into zoösporangia or resting spores. It is to be noted, however, that none of the Ancylistales, with the exception of Dangeard's (1911) *Mitochytridium*, possess so-called rhizoids which are attached to the sporangia. Furthermore the zoöspores of this group, except in *Achlyogeton* (Schenk, 1859; Martin, 1929), are all biciliate, while those of *Catenaria* are uniciliate.

In the manner of resting spore formation, however, *C. Anguillulae* is not very similar to the other species of the Cladochytriaceae. According to Buckley and Clapham (1929, figs. 1 to 21) the resting spores are formed by the retraction and contraction of the contents of the zoösporangia into a more or less spherical mass which then becomes invested with a thick, smooth, double contoured wall. This may occur even in zoösporangia which have almost reached maturity and developed long sporangial necks. The resting spores thus occupy the center of the former zoösporangia. In germination the thick wall of the spore is burst, and a small papilla gradually elongates into a neck and often extends through the old sporangial neck to the outside. In the meantime, the content of the resting spore undergoes cleavage, and the uniciliated zoöspores pass to the outside.

The position of the resting spore relative to the remainder of the thallus is very similar to that in many members of the Ancylistales, and were it not for the doubtful evidence of primitive sexuality in some species, *C. Anguillulae* might well on this basis be placed in this group rather than in the Cladochytriaceae. The evidence of sexuality in the Ancylistales so far presented (Pfitzer, 1872; Zopf, 1885; Dangeard, 1886, 1903–1906; Wildeman, 1893, 1897, etc.) is not altogether convincing, it seems to me, and until the process of oöspore or resting spore formation is better known the relation of *C. Anguillulae* to this group of fungi must remain doubtful.

If the presence of rhizoids on thread-like or elongated irregular zoösporangia is taken as a basis of relationship, *Mitochytridium* should also belong in the Cladochytriaceae near *Catenaria*. The fact that its entire thallus with the exception of the rhizoids, according to Dangeard (1911), becomes a sporangium need not exclude it, since as E. J. Butler (1928) points out this occurs occasionally in *Catenaria* also. Furthermore, the zoöspores are uniciliate as in the Cladochytriaceae. However, our knowledge of *Mitochytridium* is so fragmentary that it is premature to assign it

definitely to any particular group. A careful study of the successive stages of zoöspore germination, sporangium development, and resting spore formation may, however, reveal a life cycle very similar to that of *Catenaria*.

Fischer's inclusion of *Hypochytrium* in the Cladochytriaceae or Hyphochytriaceae as the climax genus and his view that it is a transition form to the Peronosporales have not received wide support. Schröter and Migula place it in a family of the same name but distinct from the Cladochytriaceae. Gäumann, Gwynne-Vaughan and Barnes, and Gäumann and Dodge omit it altogether, while Fitzpatrick regards it as a doubtful chytrid. None the less, if we accept Fitzpatrick's (p. 100) and Bartlett's (p. 276) characterization of the Cladochytriaceae as a group of parasitic and saprophytic fungi with a definite wide spreading mycelium on which are formed terminal or intercalary zoösporangia and asexual resting spores, *Hyphochytrium infestans* fits fairly well into this family, according to Zopf's description and figures. It is to be noted, however, that the zoöspores swim with their cilia extended anteriorly, while the so-called mycelium is comparatively large, coarse, and septate. For these reasons apparently it has been regarded as a doubtful form.

As to the genus *Rhizidiocystis* which Sideris (1927, 1929) founded for a chytridiaceous parasite, *R. Ananasi* in root hairs of pineapples, it is difficult to determine from the description and figures given whether it should be included in this family. It is to be regretted that the author did not make drawings of the successive developmental stages of the rhizo-mycelium, zoösporangia, and resting spores, since his photographs leave much to be desired. None the less, this species differs markedly from the other members of the family by the lack of zoöspores, an extremely profuse "mycelium," and rough-walled resting spores. The content of the zoösporangium does not undergo cleavage into zoöspores but is discharged through an emission canal directly into the root hair, according to Sideris. The resting spores are also described as arising from the zoösporangia. In these respects *R. Ananasi* is quite unlike any known chytrid, and it is doubtful from the author's descriptions whether this organism belongs among the Chytridiales. His figures of the "mycelium" (6 *z*) with the turbinate enlargements and zoösporangia, on the other hand, are very suggestive of the thallus of several members of the Cladochytriaceae, but until this species has been thoroughly worked over, the genus *Rhizidiocystis*, it seems to me, will remain in a doubtful position.

Summary

1. *Cladochytrium replicatum* n. sp. occurs in dying and decaying cells of *Eriocaulon septangulare*, *E. compressum*, *Elodea canadensis*, *Myriophyllum verticillatum*, *Cladophora glomerata*, and *Spirogyra* sp. As far as present observations go it causes no hypertrophy or malformation of the host plant and appears to be weakly parasitic.

2. Its vegetative thallus consists of a tenuous, branched rhizo-mycelium

with one- to several-celled turbinate organs which is replicated to a large extent as it spreads in the host tissue. Intercalary and terminal, spherical, oval, and irregular zoösporangia of variable diameter occur at various intervals on the rhizo-mycelium. The zoöspores, 4–7 μ in diameter, are discharged through a sporangial neck and lie quiescent in a mass for a few seconds before swimming away. Each zoöspore possesses a single posteriorly attached cilium and a brilliant golden brown globule.

3. The vegetative thallus of the Cladochytriaceae is designated as a rhizo-mycelium with the view of calling particular attention to its difference from the rhizoidal system of the Rhizidiaceae.

4. Large oval, spherical, and irregular golden zoösporangia which may occupy as many as thirteen host cells and produce hundreds of zoöspores have often been found in cells of *Eriocaulon*, *Elodea*, and *Myriophyllum*. Resting spores have so far not been found.

5. Whether or not the forms which occur on the various host plants are the same species is uncertain, but the evidence at hand suggests that they constitute a single ubiquitous species which parasitizes numerous aquatic plants. Cross-inoculation experiments are now in progress.

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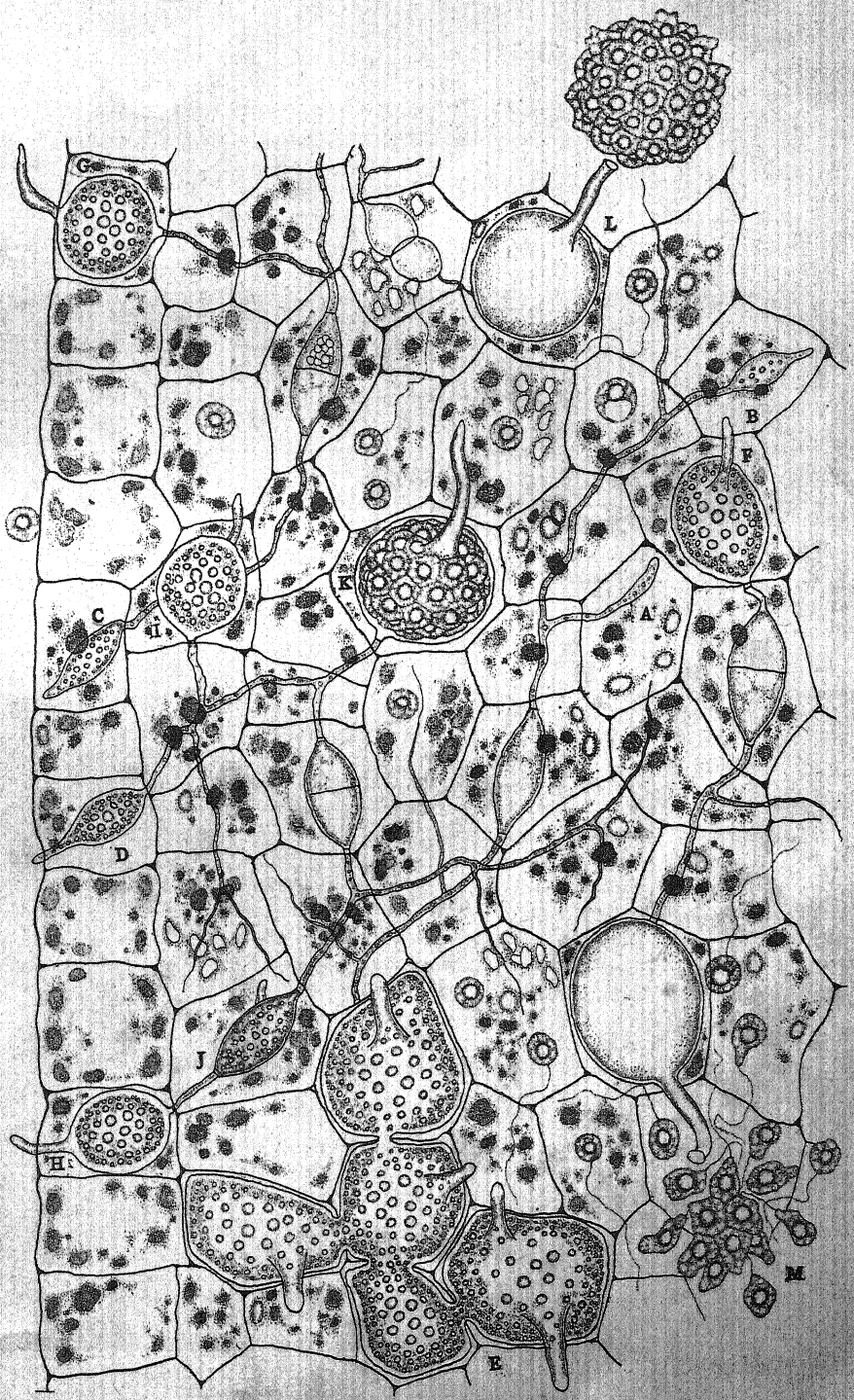
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EXPLANATION OF PLATES

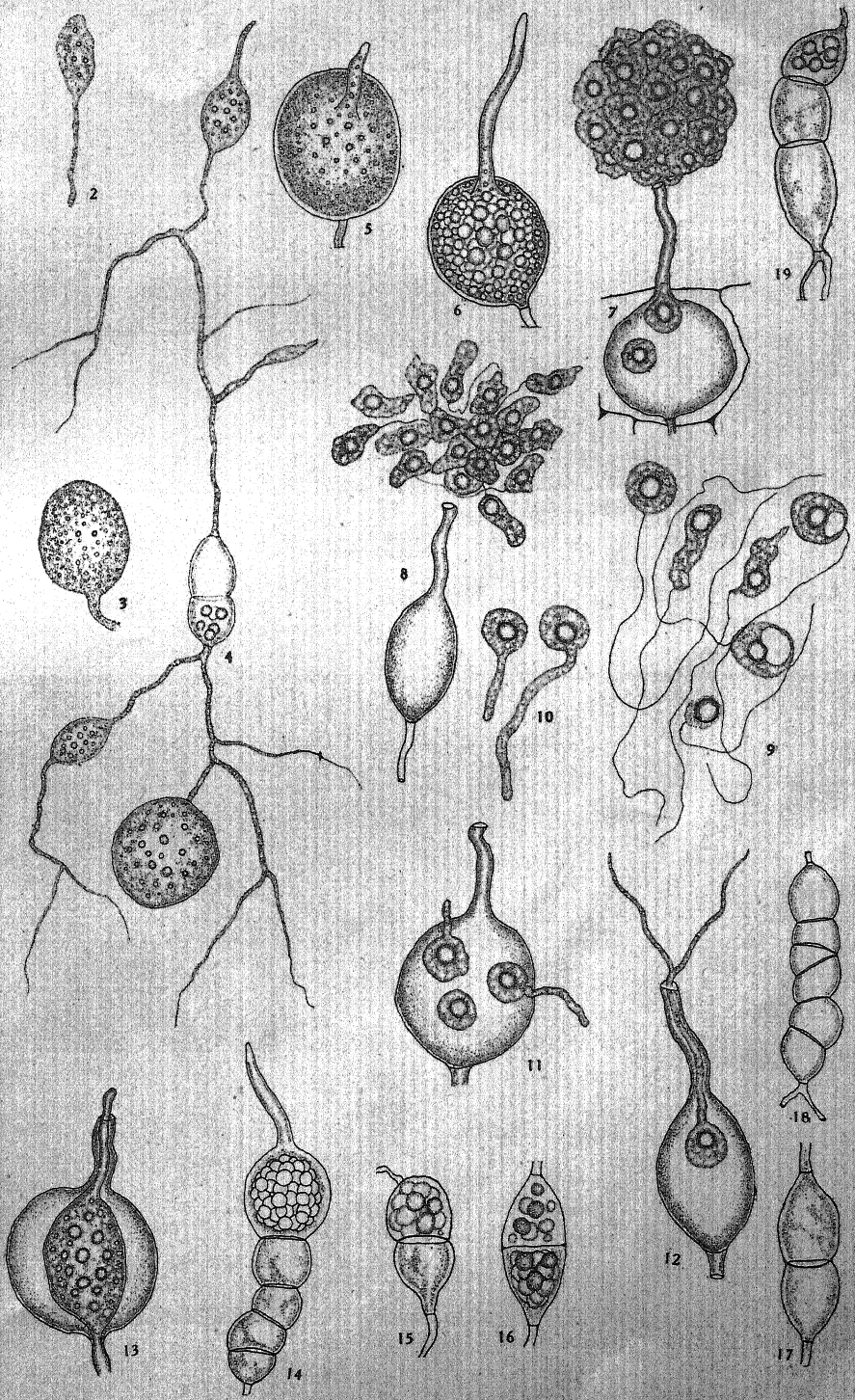
All figures were drawn from living material with the aid of a Zeiss camera lucida, a Zeiss 2 mm. apochromatic objective N.A. 1.30 and compensating oculars numbers 6 and 8.

PLATE XLII

FIG. 1. A portion of a leaf of *E. septangulare* showing the profuse occurrence and general habit of the rhizo-mycelium, turbinate organs and zoösporangia of *Cladochytrium*

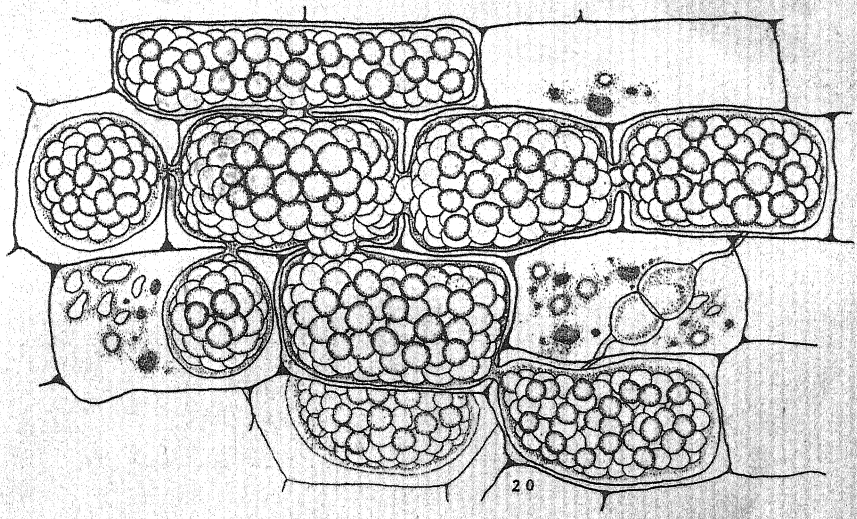


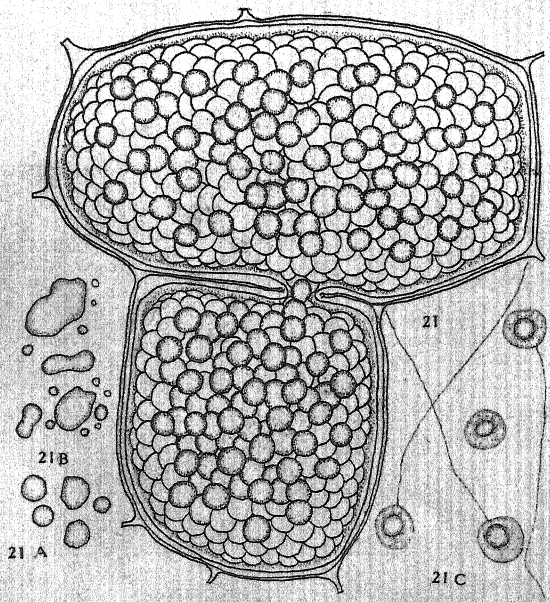
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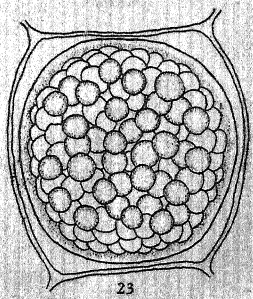


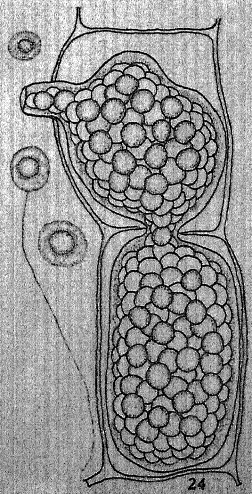
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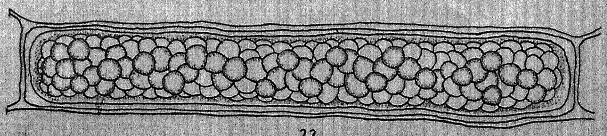












KARLING: CLADOCHYTRIUM

replicatum. The host cells are filled with degenerating plastids, starch grains and other types of bodies and granules. *A*, *B*, *C*, *D*, *F*, *G*, *H*, *I*, *J*, *K*, *L*, and *M*, stages in the development of the zoösporangia and zoöspores. *E*, a large irregular zoösporangium which occupies five host cells.

PLATE XLIII

FIG. 2. An early stage in the development of a terminal zoösporangium.

FIG. 3. A later stage.

FIG. 4. The general appearance of the so-called rhizo-mycelium with a turbinate organ and three incipient zoösporangia. The branches run out to extreme fineness.

FIG. 5. A further stage in the development of the zoösporangium, which has become invested with a definite membrane and developed a short neck. Numerous small golden brown granules or droplets are present in the cytoplasm.

FIG. 6. A later stage in which the sporangial neck is greatly elongated and the golden bodies occur in great abundance.

FIG. 7. The zoöspores have escaped en masse, with the exception of two, and are lying quiescent at the mouth of the sporangium.

FIG. 8. The zoöspores are beginning to disperse.

FIG. 9. A group of zoöspores with long cilia and a single golden brown body in the center of the cell. Two zoöspores are amoeboid in shape, and three are undergoing degeneration.

FIG. 10. Two successive stages of zoöspore germination. The penetration tubes in this instance are quite thick and blunt.

FIG. 11. Three zoöspores which remained in the zoösporangium, two of which have germinated.

FIG. 12. A single zoöspore which has germinated *in situ*, the penetration tube having passed out through the sporangial neck.

FIG. 13. A proliferating zoösporangium.

FIGS. 14–19. Variations in the size, shape, content and septation of the turbinate organs.

PLATE XLIV

FIG. 20. A large irregular zoösporangium which has spread into nine adjacent host cells. The entire sporangium is filled with golden brown predominantly spherical bodies.

FIG. 21. A similar zoösporangium from *E. compressum* occupying two cells, which appear somewhat distended by the presence of the chytrid.

FIG. 21 *a*. Variations in the size and shape of the golden brown bodies from a single zoösporangium.

FIG. 21 *b*. Changes in size and shape which these bodies undergo with pressure on the cover glass. They may become dispersed or flow together into larger masses.

FIG. 21 *c*. Zoöspores from irregular zoösporangia.

FIG. 22. A large zoösporangium in an elongated cell of *E. compressum*.

FIG. 23. A large spherical zoösporangium from *M. verticillatum*.

FIG. 24. An irregular zoösporangium from cells of *Elodea canadensis*.

PLANT ANATOMY AS CONDITIONED BY LIGHT INTENSITY AND SOIL MOISTURE[1]

WILLIAM T. PENFOUND

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INTRODUCTION

The effect of physical factors on the structure of leaves has long been a popular subject for investigation. Most of the researches have been concerned with plants growing under field conditions, only a few having dealt with the effect of a given factor under controlled conditions. Furthermore, few of the studies on ecological plant anatomy have been concerned with the structure of roots and stems and few experimental data are available on those plant parts. An attempt has been made in the present investigation to determine the effects of varied light intensity and soil moisture on the structure of roots, stems, and leaves of selected species of plants.

Isolation of a factor such as light intensity is difficult under field conditions since low air temperature and high relative humidity are usually associated with a low light intensity. However, it has been found possible to obtain a variation in light intensity without appreciably affecting either the air temperature or humidity by means of a specially constructed shade shelter. The leaf temperature is nevertheless affected, a difficulty thus far impossible to overcome. Modification of water content is more easily attained without greatly affecting other physical factors. It is to be noted, however, that the maintenance of an even distribution of water in a comparatively dry soil is very difficult. This is especially true since roots may absorb water in their immediate vicinity faster than the soil can supply water to them.

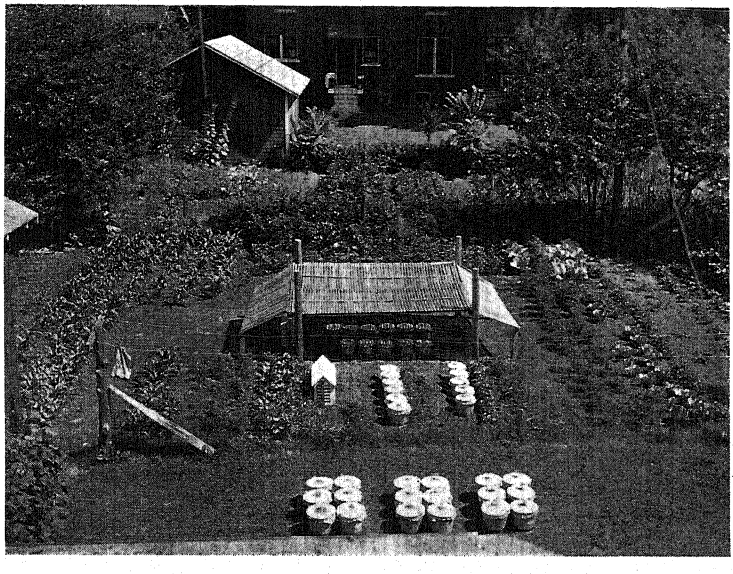
An experiment in the summer of 1926 dealing with the effect of light intensity was conducted in a clearing at the southeast corner of the "University Woods" near Urbana, Illinois. A later one was conducted in a garden in Urbana in the summer of 1927. The preliminary soil moisture studies were made in the University of Illinois greenhouses in 1926 and a more detailed investigation was carried on in a garden at Urbana in the summer of 1927.

EXPERIMENT ON THE EFFECT OF LIGHT INTENSITY IN WHICH PLANTS WERE WATERED DURING THE GROWTH PERIOD

Plants were grown during the summer of 1926 in full sunlight and under a lath shelter and their structure later compared (text fig. 1). The shelter

[1] Contribution from the Botanical Laboratory of the University of Illinois.

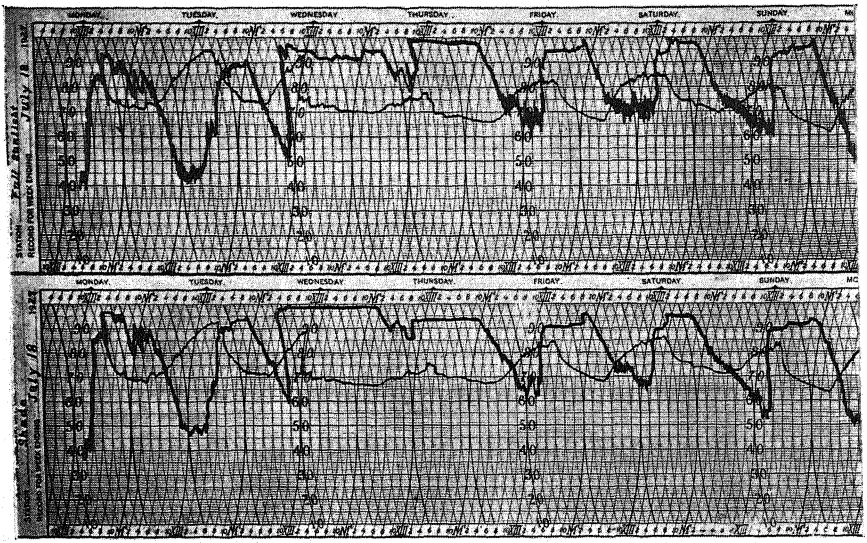
consisted of three frames covered with lath strips 1½ inches apart. The largest frame (8 × 10 ft.) was supported horizontally 3½ feet above the soil surface by four uprights and the two other frames were placed at the ends of the large frame at an angle of 40 degrees with the uprights in order to prevent the full sun of morning and evening from shining on the plants.



TEXT FIG. 1. Shelter, hygrothermographs, and batteries of plants.

All vegetation was removed from under the shelter; two trenches six inches deep running lengthwise under the shelter were made and the entire soil surface was covered with two inches of cinders. The light intensity, as calculated from an average of hourly readings from 7 A.M. to 6 P.M. and based on mid-day sun on June 22, was 18% for the shelter and 90% in the full sunlight. Due to the absorption of radiant energy by the cinders and to the height of the 'roof' above the soil surface the shelter had no appreciable effect on air temperature or relative humidity (text fig. 2).

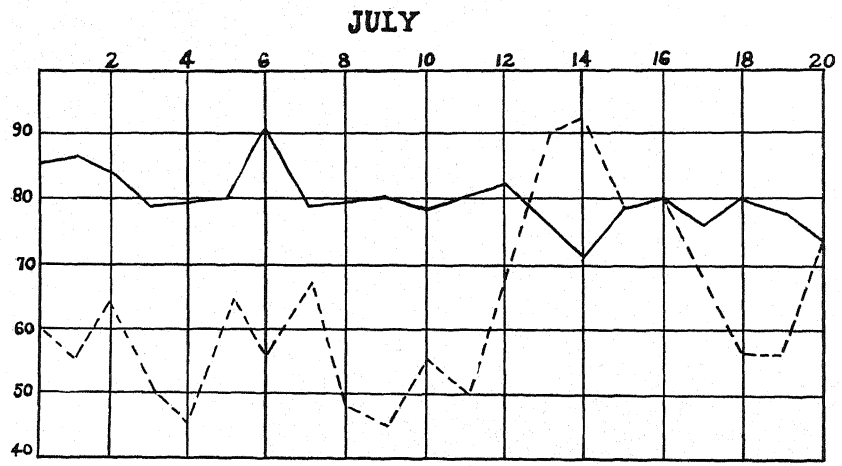
Five plants each of *Helianthus annuus* L. (Mammoth Russian sunflower) and *Polygonum hydropiper* L. (Water pepper) were grown from May 28 to June 25, 1926, in 14-liter galvanized buckets containing a black silt loam with a water content of 24% as based on the dry weight of the soil. The individual potometers were sealed with oil-cloth and were weighed weekly, at which time water was added by way of watering tubes to bring them to their original weights. The average total transpiration per plant for a *Helianthus* was 3610 cc. in the sunlight and 821 cc. in the shade and for *Polygonum* it was 3125 cc. in full sun and 1190 cc. under the shelter. In addition the average weekly transpiration per plant increased more rapidly in the full sunshine than it did under the shelter.



TEXT FIG. 2. Hygrothermograph records from full sunlight (above) and the shelter (below) for the week ending July 18, 1927.

Light lines, temperature; heavy lines, humidity.

The plants grown in full sunlight were more robust and produced more leaves but the heights of the plants in both conditions were similar. Leaves of *Helianthus* were much larger in full sun whereas the leaves of *Polygonum* were slightly smaller. Although the leaf size varied with the species the number of stomata per unit area was always higher in the sunlight. The root system was more extensive and the roots thicker in the condition of

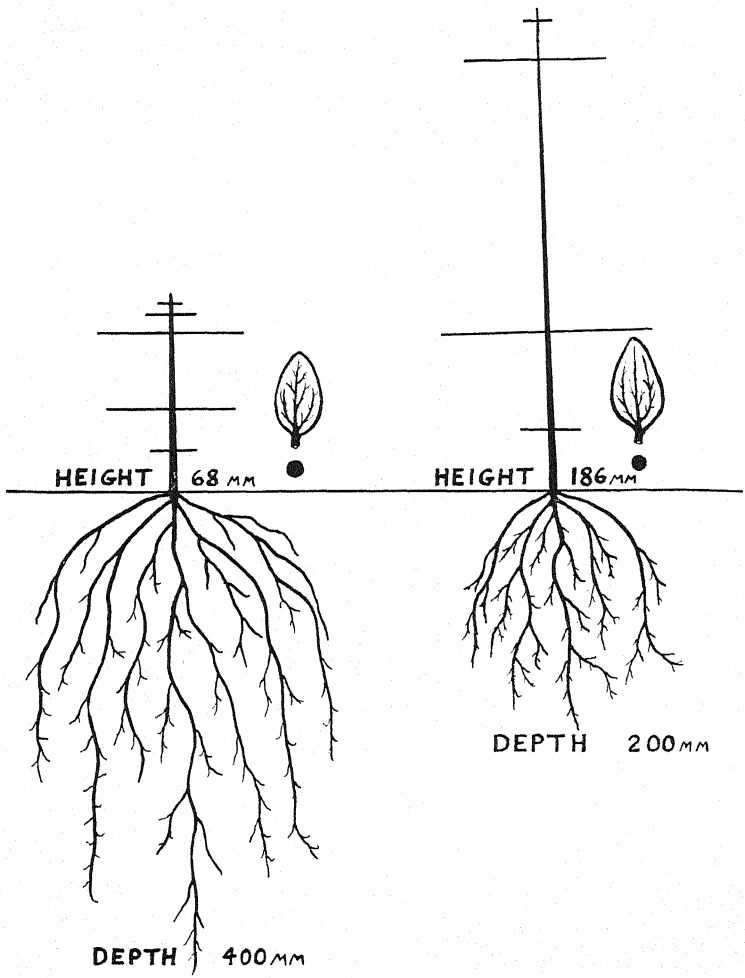


TEXT FIG. 3. Average daily temperature (solid line) and average daily humidity (broken line) during growth period in the summer of 1927.

maximum insolation. Since the results on comparative structure were similar in this and the following experiment the data in this study are not reproduced here.

Experiment on the Effect of Light Intensity in Which Plants Were Grown Without Additional Water

In the summer of 1927 a more detailed experiment was conducted in a garden at Urbana, Illinois. Plants of *Helianthus annuus* L. (Mammoth Russian sunflower) were grown for three weeks in containers of large size in full sunlight and under a shelter without the addition of water. The shelter and the condition of the soil surface were the same as in the previous



Text Fig. 4. Relative development of the sunflower grown in full sunlight (left) and under a shelter (right).

experiment. The containers were prepared as follows. Thoroughly sieved and mixed soil with a water content of 24% and a wilting coefficient of 14% (based on dry weight of soil) was alternately added and compacted into 14-liter containers until they were filled. To these containers seedlings one inch in height and of similar vigor were transplanted, the soil surface being sealed with oil-cloth. One set of six potted sunflowers was then placed in a trench six inches deep in full sunlight and six others were set in the trenches under the shelter on July 4 and allowed to grow until July 25 (text fig. 1).

The light intensity, as calculated from an average of hourly readings from 7 A.M. to 6 P.M. and based on mid-day sun on June 22, was 77.5% in full sunlight and only 16% under the shelter. During the growth period the average daily temperature did not vary greatly but the average daily relative humidity often changed rapidly from day to day (text fig. 3). But both air temperature and relative humidity in full sunlight and under the shelter were similar (text fig. 2).

External Features

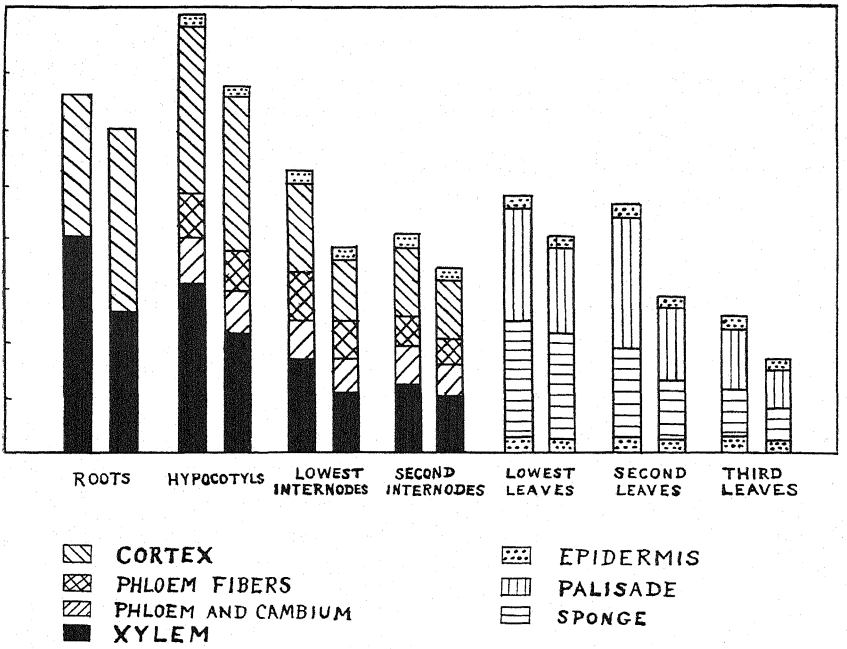
In spite of the similarity of environmental conditions the average total transpiration per plant was greater and the development of the plants was better in full sunlight than in shade (text fig. 4). The roots were thicker and longer, the hypocotyls and internodes had a greater diameter and the leaves were thicker and more numerous. The plants grown in sunlight possessed many roots between 12 and 16 inches in length whereas those grown in shade had no roots over 10 inches long. But the height of the shoots was greatest under the shelter. Although leaf size was similar in both situations the number of stomata per unit area was 85% greater for the upper epidermis and 75% more for the lower epidermis in full sunlight. On a given plant the stomata were more numerous per unit area and smaller at successively higher nodes.

Internal Features

The plant parts were sectioned, stained in safranin and light green, and studied quantitatively. A study of the roots, as based on sections of the main laterals one-third the distance from the main root, showed that those from sun plants were thicker, had a much larger xylem area, and larger xylem elements (text fig. 5). This increased development of xylem is in direct relation to the increased rate of water-loss by the sun plants. In the hypocotyls (text fig. 5) the most noticeable difference was the much greater mass of xylem from plants developed in sunlight. This was due primarily to a greater amount of secondary thickening. In addition the percentage area of xylem, as compared to the cross-sectional area, was greater in the exposed habitat. Since a greater proportion of the elements in the xylem were wood fibers and since the phloem fibers were more

numerous and thicker-walled the sun plants showed better mechanical development.

The internodes of plants developed in full sunlight possessed a greater total diameter, a greater radial dimension of all regions, especially of the xylem, and more rows of collenchyma than those grown in shade (text fig. 5). Although the plants grown in full sun were less than one half as tall as the shade plants the length of the component cells was similar in both. Thus the increased height of the shade plants was due to an increase in number of cells and not to an increase in the length of the cells along the vertical axis.



TEXT FIG. 5. Relative depths of regions in sunflower plants grown in full sunlight (left) and under a shelter (right). Each space on the vertical scale equals 100 μ for the roots and leaves and 200 μ for the hypocotyls and internodes.

Only the lowest leaves of both sets of plants were the same age at the end of the experiment but all the leaves from corresponding nodes of both sets of plants showed the same type of variation in structure (text fig. 5). The leaves of plants grown in sunlight were thicker, the palisade and spongy chlorenchyma were deeper, the epidermal cells over the midvein were larger, and all regions of the midvein (notably the collenchyma) showed greater dimensions. The youngest leaves on both sets of plants were very similar and consisted of six layers of nearly cubical cells without evident air spaces. On the other hand the leaves at successively lower nodes on the same plant often differed more from each other than did leaves at corresponding nodes from plants grown in full sunshine and in shade.

In the development of plants in full sunlight and in shade the interrelations of the absorbing, conducting, and transpiring areas are often of vital importance. Although the areas of the root systems were not determined in this experiment, the data (text fig. 4) amply demonstrate the fact that the ratio of the root area to the leaf area was higher in full sunlight than under the shelter. In both the hypocotyls and the lowest internodes the ratio of the vascular area to the leaf area was higher in the more exposed situation (table 1).

TABLE 1. *The Effect of Light Intensity on the Relations Between the Vascular and Transpiring Systems in the Sunflower*

	Sun	Shade
$\frac{\text{Xylem area of lowest internode}}{\text{Transverse area of lowest internode}}$	9.5%	7.0%
$\frac{\text{Vascular area of lowest internode}}{\text{Average leaf area per plant}}$	0.20%	0.12%
$\frac{\text{Transpiration}\quad \text{Av. per plant}}{\text{Cc. per day}\quad \text{Av. per sq.dm.}}$	$\frac{9.5}{11.8}$	$\frac{6.0}{6.4}$
$\frac{\text{Av. transp. per plant per day}}{\text{Xylem area of lowest internode}}$	19.8	20.0
$\frac{\text{Av. transp. per plant per day}}{\text{Vascular area of lowest internode}}$	58.5	61.0

In spite of these facts, the ratio of the average transpiration per plant per day to the vascular area in both the hypocotyls and lowest internodes was approximately the same in both sets of plants. This means that the amount of water passing over a unit vascular area in a unit of time was similar in both conditions of insolation. In the lowest internodes the amount of water passing over one square millimeter of vascular tissue per day was 58.5 cc. in the sun plants and 61.0 cc. in the shade plants. Thus, in spite of their poorer root system and smaller conducting area, the shade plants, as long as they are kept in the shade, appear to be just as well equipped in their water balance as the plants in full sunlight.

PRELIMINARY EXPERIMENTS ON THE EFFECT OF VARIED SOIL MOISTURE CONTENT

In the winter and spring of 1926 six species of plants were grown in soil masses of widely divergent water content in one of the University of Illinois greenhouses, and their structure later compared. The plants were potted in 8-inch porous pots containing a black silt loam soil. All the potted plants were placed in 10-inch graniteware pans and water was added to the pans to keep one set continuously saturated, to maintain another set with medium amount of water, and to furnish another set with only enough water to keep them from wilting daily.

In the winter three potted plants each of Mammoth Russian sunflower, Marquis spring wheat, and Wardwell's kidney wax beans were grown in the above conditions for a period of 24 days. In the spring three potted plants each of *Helianthus scaberrimus* Ell., *Laportea canadensis* (L) Gaud., and *Lespedeza capitata* Michx. were grown for a period of 49 days in continuously saturated soil and in alternately moist and dry soil. In the latter experiment the plants growing in dry soil wilted from six to ten times during the growth period but regained turgidity upon the addition of water. On the other hand no plants wilted in soil of high water content.

At the end of the growth period the development of the root system, the average height and diameter of the stems, the number and size of the leaves, the size of the stomata, and the stage of reproduction varied directly as the amount of water applied. Since the data in these preliminary experiments on the effect of soil moisture were similar to those in the more detailed experiment in 1927 only the general results are given here. Plants of all species grown in soil of high water content differed from those in relatively dry soil as follows. The roots were thicker, showed a greater amount of secondary thickening, had more and larger xylem vessels and thicker-walled wood fibers. The stems were thicker, showed greater actual and percentage areas of xylem, possessed more and larger vessels and had thicker-walled phloem and wood fibers. In the leaves the palisade and spongy chlorenchyma were deeper and thus the leaves were thicker. Increased soil moisture, therefore, augmented both the size and thickness of the leaves.

Detailed Experiment on the Effect of Soil Moisture Content

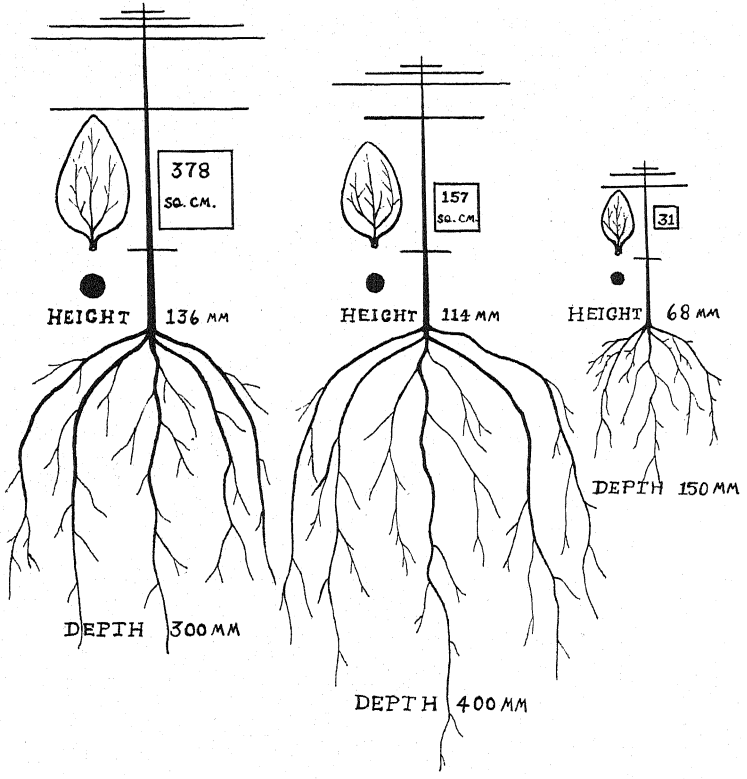
A more detailed experiment on the relation of the amount of soil moisture to plant structure was conducted in a garden at Urbana, Illinois, in the summer of 1927. Sunflower plants were potted in buckets of 14-liter capacity containing thoroughly mixed and sieved soil of diverse water content and allowed to develop without further addition of water. Three sets of six containers each were prepared, the available water contents (based on dry weight) being 21%, 12%, and 4%. On June 28 seedlings of Mammoth Russian sunflower of similar vigor and one inch in height were transplanted to the containers and the soil surfaces were sealed with two layers of oilcloth. The potted plants were transferred to an open lawn on June 29 and allowed to grow for 18 days. During the growth period the average daily air temperature was fairly constant but the average daily humidity fluctuated widely (text fig. 2). The average total water loss per plant in wet soil was 322 cc., in moist soil 200 cc., and in dry soil only 80 cc. In addition plants in moister soil showed a greater increase in the daily transpiration per plant as growth proceeded. On the other hand, the transpiration per unit area was greater the drier the soil.

The plants growing in wet soil and in moist soil showed no signs of wilting whereas the plants in dry soil wilted every afternoon toward the end of the

growth period. It was necessary to terminate the growth period on the 18th day which was much earlier than was planned. But at the end of the growth period a determination of available water at a depth of 0 to 5 inches showed 16% for the wet soil, 10% for the moist soil, and 3% for the dry soil. The results suggest that mesic plants, such as the sunflower, need a considerable percentage of available water in order to thrive in an atmosphere of relatively high evaporating power.

External Features

The development of the plants at the end of the growth period was in direct ratio to the water content of the soil (text fig. 6). The average leaf

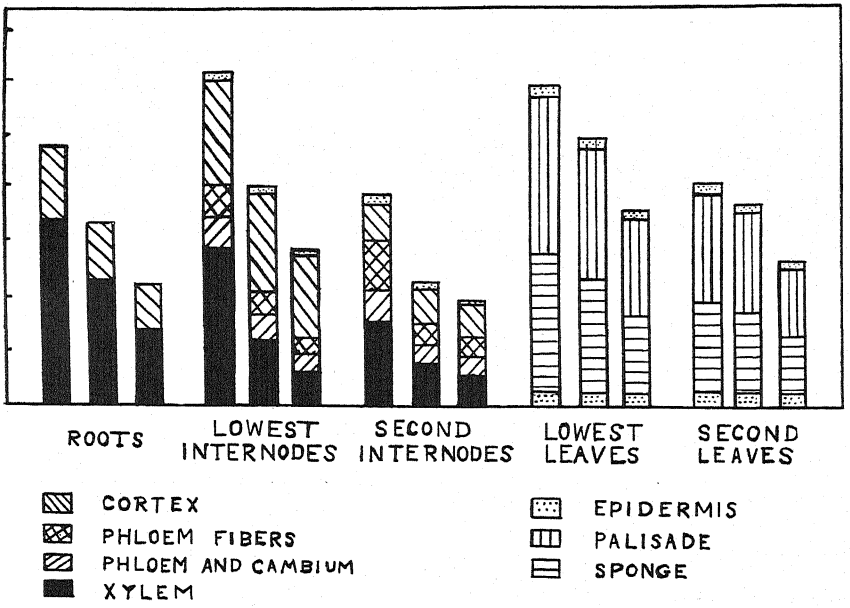


TEXT FIG. 6. Relative development of the sunflower grown in wet soil (left), moist soil, and dry soil (right).

area of the wet soil plants was especially notable, being more than twelve times that of the dry soil plants. Only three roots of plants growing in wet soil reached a length of 12 inches whereas one or more roots of every plant growing in moist soil attained or exceeded this length. But no roots of the plants grown in dry soil reached deeper than six inches and most of them were confined to the upper four inches of soil.

Internal Features

The selection of roots for comparison of their structure is a considerable problem since it is very difficult to tell the exact age of any portion of the root system. The results obtained were based on root sections taken from the main laterals two centimeters from the primary root. They showed that the diameter of the root, the depth of each region, the amount of secondary thickening, and the number and size of the water-conducting



TEXT FIG. 7. Relative depths of regions in sunflower plants grown in wet soil (left), moist soil, and dry soil (right).

vessels was directly related to the percentage of available moisture (text fig. 7).

The comparative data on the structure of hypocotyls and internodes of the sunflower indicate that they are fully as sensitive as are the leaves to variation in soil moisture. In the hypocotyls the total thickness, the radial dimension of each region, the size of the component cells, the wall thickness of the mechanical elements, and the number and size of the water conducting vessels varied with the percentage of available moisture. In addition the actual and percentage areas of the xylem, as determined with the aid of a microprojector and a planimeter, were greater the more moist the soil (text fig. 8).

In the lowest internodes many differences in structure were directly related to a higher water content (text fig. 7). The internodes were thicker, the depth of all regions was greater, the phloem and wood fibers were more abundant and thicker-walled, and the water conducting vessels were larger

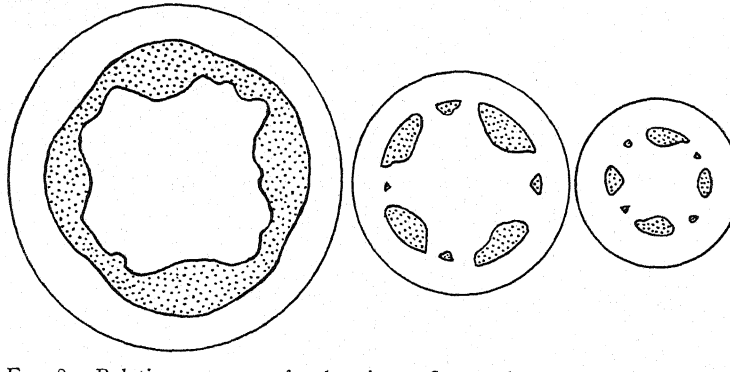
and more numerous. As in the hypocotyls both the actual and percentage areas of xylem varied directly with the percentage of soil moisture.

In leaves at corresponding nodes the size of the stomata varied in the same direction as the water content. On the other hand the number of stomata per unit area was greater by 50% in the dry soil plants and by 33% in the moist soil plants when compared to that in the wet soil plants. The thickness of the leaf, the depth of the palisade and spongy chlorenchyma, the size of the epidermal cells, and the number and size of the xylem vessels varied directly as the percentage of soil moisture (text figure 7). But the number of rows of palisade and sponge was constant in all sets of plants. Since the cell size in the leaves varied in the same direction as the soil moisture content, the observed differences were apparently due to this fact.

The youngest leaves in all sets of plants were very similar and consisted of six layers of nearly cubical cells without air spaces between them. On the other hand, leaves at successively lower nodes on the same plant often showed greater differences than leaves from corresponding nodes of plants grown in soil of different water content. Since development proceeded to the end of the experiment (18 days) and since plants in wet soil developed most rapidly it is probable that the observed differences were mainly due to this diversity in developmental rate. It also appears likely that dry soil plants actually have less time for development due to inactivity, during the wilted or partly wilted condition, in periods of high evaporational stress.

Relation of Structure to Water Balance in the Sunflower

In heliophilous plants the interrelations of the area of the root system, the conducting area of the stem, and the leaf area are of greatest importance. Although the surface areas of the root systems were not determined in this



TEXT FIG. 8. Relative amounts of xylem in sunflower plants grown in wet soil (left,) moist soil, and dry soil (right).

experiment the data on the diameter, length, and number of roots suggest that the ratio of root area to leaf area is greater the drier the soil. It is, therefore, probable that plants growing in drier soil possess a more efficient

absorbing system relative to their transpiring surface. In both hypocotyls and the lowest internodes the ratio of the xylem area to the leaf area was higher the drier the soil. This was true also for the ratio of the actual conducting area to the transpiring area (table 2). In other words, the drier the soil, the smaller the transpiring surface served by a unit area of vascular tissue.

TABLE 2. *The Effect of Soil Moisture on the Relations between the Conducting and Transpiring Systems in the Sunflower*

	Wet	Medium	Dry
$\frac{\text{Xylem area of lowest internode}}{\text{Average leaf area per plant}}$	0.39%	0.43%	0.55%
$\frac{\text{Vascular area of lowest internode}}{\text{Average leaf area per plant}}$	0.14%	0.16%	0.24%
$\frac{\text{Transpiration}}{\text{Cc. per day}} \quad \frac{\text{Av. per plant}}{\text{Av. per sq.dm.}}$	$\frac{17.9}{4.7}$	$\frac{11.1}{8.1}$	$\frac{4.4}{14.2}$
$\frac{\text{Av. transp. per plant per day}}{\text{Xylem area of lowest internode}}$	12.0	19.0	26.0
$\frac{\text{Av. transp. per plant per day}}{\text{Vascular area of lowest internode}}$	33.8	50.5	58.0

In spite of these facts the ratio of the average water loss per plant per day to the conducting area was higher the drier the soil. This means that the amount of water passing over unit xylem or vascular area in a unit of time varied directly as the soil aridity. Thus in the lowest internodes the amount of water passing one square millimeter of xylem per day was 33.8 cc. in the wet soil plants, 50.5 cc. in the moist soil plants, and 58.0 cc. in the dry soil plants. The explanation of this increased flow of water in the plants grown in drier soil lies in the higher transpiration rate of these plants.

DISCUSSION AND CONCLUSIONS

The results of many investigations have shown that xeromorphic structure is closely associated with an arid habitat. In addition Maximov (1929) showed that plants growing in arid regions had a high osmotic pressure, a rapid transpiration rate, and an efficient rate of carbon assimilation. It was shown by Zalenski (Maximov, 1929) that xeromorphism increased with the distance of the leaves from the root system. His results suggest that strong insolation and dry air are both effective in inducing xeromorphic structure. These conclusions were supported by the work of Maximov (1925, quoted in Maximov 1929) and Eberhardt (1903).

In the experiments here presented the plants grown in full sunlight and in dry soil showed pronounced xeromorphism; but in dry soil the mechanical

elements were fewer and thinner-walled than in more moist soil. The same results were obtained by Kohl (1886), Haberlandt (1914), and Rippel (Maximov, 1929). Barss (1930) showed that the pear tree produced a greatly increased volume of firm, sound wood in soil of high water content. These results suggest that a high soil moisture content favors mechanical development, but before definite conclusions can be drawn very careful studies must be made on the combined area of all the mechanical elements in the stem relative to the total leaf area.

The question of the effect of soil moisture on vascular development is still unsolved. Cannon (1905), and Rippel (Maximov, 1929) found a better development of vascular tissue in dry soil whereas Pohl (1926) found wider and larger vessels in conditions of high water content. Barss (1930) stated that the amount of xylem and the size of the vessels was greater the wetter the soil. Yapp (1912) found that the leaf cells were larger the higher the water content of the leaves. In my experiments all the cells were larger, and the percentages of xylem, and of vascular area, were greater the wetter the soil.

The relations existing between the conducting and transpiring areas in plants have been studied by Rübel (1920), and Huber (Maximov, 1929). In sun and shade plants of the sunflower Rübel found that the ratio of the vascular area of the stem to the transpiring surface above the cross-section was nearly a constant. The ratio was 0.03% higher in plants grown in full sunlight whereas my results show a difference of 0.08% in favor of the sun plants. In the experiments of Rübel and Huber the ratio of the intensity of transpiration to the actual vascular area of the stem was higher in shade plants than in sun plants. It should be pointed out, however, that the rate of flow is not concerned with the intensity of transpiration but is found by dividing the actual transpiration per plant in a given period by the vascular area in a designated internode of the stem. In my experiments the ratio of the intensity of transpiration to the vascular area was somewhat lower in the shaded condition. The rate of flow, however, was approximately the same in both sets of plants indicating that sun and shade plants are probably equally efficient in regard to water conduction.

In the soil moisture experiments the ratio of the total transpiration per plant to a unit vascular area was higher the drier the soil. In addition, the ratio of the intensity of transpiration to the vascular area was still higher the more arid the soil. These results suggest that the moisture content of the soil may be a more important factor than light intensity in determining the relationships existing between water conduction and water loss.

In the present studies certain results were obtained which are paradoxical or run counter to current opinion. The leaves of *Helianthus* in full sunlight were larger than those of plants growing under the shelter whereas the reverse was true for *Polygonum*. But when the sunflower was not

watered during the growth period the leaves of the sun plants were smaller than those of the shade plants. In the sunflower the cells of the stem were longer in the sun plants although the shade plants were much taller. The number of stomata varied with the degree of insolation although the epidermal cells in both sets of plants were equal in size.

In the soil moisture series the number and wall thickness of the mechanical elements varied directly with the soil moisture content. In addition the percentage area of xylem was always higher the wetter the soil. But, despite this fact, the dry soil plants showed a more efficient conducting system per unit area of leaf surface. In all experiments the young leaves were similar in structure but successively lower leaves differed greatly from one another. Another unexpected result was the fact that the thickness of the leaves varied in the same direction as the water content of the soil.

Summary

Plants grown in full sunlight differed from those grown in shade as follows:

1. The roots had much more xylem and a greater diameter.

2. The hypocotyls showed a greater percentage area of xylem, an increased diameter, and more and thicker walled mechanical elements.

3. In the internodes all regions were deeper, the total diameter was greater, and the component cells were relatively longer when compared with the height of the plant.

4. In the leaf the epidermal cells were larger, the stomata were smaller but more numerous per unit area and all regions, both in the mesophyll and midvein, were larger.

5. The ratio of the vascular area to the leaf area was higher but the rate of flow in the vessels was similar.

Plants grown in soil of high water content differed from those grown in dry soil as follows:

1. The roots possessed more xylem, more and larger water-conducting vessels, and an increased total diameter.

2. The hypocotyls showed a greater percentage area of xylem, a larger size of all cells, and more and thicker-walled mechanical elements.

3. The internodes showed a greater depth of all the regions, a greater percentage area of xylem, a larger size of all cells, and more and thicker-walled phloem fibers.

4. In the leaves the stomata were larger but less numerous per unit area, all regions in the mesophyll and midvein had greater dimensions, and the leaves were, therefore, thicker.

5. The ratio of the vascular area to the leaf area was lower as was the rate of flow in the vessels.

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PRELIMINARY STUDY OF CHEMICAL PROCESSES INVOLVED IN THE DECOMPOSITION OF MANURE BY *AGARICUS CAMPESTRIS*[1]

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The common edible mushroom, *Agaricus* (*Psalliota*) *campestris*, is usually grown for commercial purposes on a compost of horse manure which has first been allowed to decompose, for a period of about four to six weeks, in loosely kept and frequently mixed composts. Certain chemical changes are brought about in the manure, in the process of composting, by its natural flora of bacteria and fungi; these changes result in making the manure a better medium for the growth of the cultivated mushroom. The nature of these changes as well as the nature of the chemical complexes upon which the fungus draws for its nutrition are still imperfectly understood. Since the mushroom is an heterotrophic organism, it must derive not only its nitrogen and mineral elements from the manure compost, but also its carbon. In view of the fact that the composted manure is not a single chemical compound but a mixture of numerous chemical complexes, it still remains to be determined whether the mushroom-fungus attacks all the complexes at the same time, or is able to utilize only some constituents of the manure in preference to others. These are of course used to supply energy to the organism as well as carbonaceous and other nutrients essential for the synthesis of the very extensive mycelium within the compost and finally of the fruiting bodies above it.

Investigators did not agree upon an explanation of the fact that organic waste products must undergo first a process of "fermentation" or decomposition before the mushroom is able to derive from it the necessary nutrients. It was usually assumed (6) that all the soluble organic matter must first be destroyed by the action of bacteria. Duggar (1) has shown that this preliminary "fermentation" is totally unessential for mycelial growth. In pure culture, the mycelium of the mushroom grows slowly but later develops abundantly upon fresh manure, even much better than on well-fermented manure. However, under commercial conditions, the rapid growth of bacteria and fungi upon the fresh manure causes such a rise in temperature that it proves injurious to the development of the mushroom mycelium. Rapid development of bacteria and the numerous manure-inhabiting fungi will also prove otherwise injurious to the development of the mushroom mycelium.

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For practical purposes, therefore, the manure must be first composted before the mushroom spawn is introduced. Voelcker (11) was the first to make a study of the changes taking place in manure in the process of composting. He found that there was at first an increase in the soluble organic matter and nitrogen, followed by a decrease; the insoluble organic matter was found to decrease gradually, while the inorganic matter gradually increased. The chemical composition of the manure was influenced by the nature of the animal diet, the kind of bedding, the care of the manure, the work done by the animal, etc.

According to Hébert and Heim (4), the composting of horse manure, for the purpose of making it a favorable medium for the growth of the mushroom brings about a series of changes in the chemical composition of the manure. There is a decided loss in the ammonia, the fatty substances, gums, tannins and especially the celluloses and pentosans (especially the xylans or "straw-gum"). These losses are accompanied by an increase in the nitrogenous complexes and in the vasculose (the earlier French name for the complex which is now known as lignin), which constitute the black matter of the manure. The growth of the mushroom on this compost leads to further losses of organic matter in the manure, especially the nitrogen compounds and the minerals, which are due to the partial assimilation of these compounds by the mushroom and partly to leaching.

According to Falck (3), one must distinguish two types of manure decomposition: 1, "hot compost," in which the celluloses are decomposed, in addition to the soluble carbohydrates and proteins, while the lignin accumulates quantitatively; 2, "nitric acid or dissimilative humification," in which both the lignin and cellulose are decomposed. The composting of manure for the growth of the mushrooms belongs to the first process. He speaks further of the mushroom fungus as a lignin-destroying form.

Waksman and Diehm (13) have also shown recently that in the composting of manure, the pentosans and cellulose are decomposed much more rapidly than the total organic matter, while the lignins are attacked less rapidly, thus leading to the reduction of the polysaccharides and accumulation of the lignin; parallel with these processes there is a rapid increase in the proportional content of organic nitrogenous complexes.

The process of composting of manure for the growth of mushrooms accomplishes several ends: it removes the soluble organic matter in the manure which is injurious to the growth of the mushroom; it removes a large number of organisms that were brought into the manure and are capable of making extensive growth on the water-soluble substances; it removes the readily decomposable complexes, thus preventing other organisms from making abundant development upon the composted manure; it makes the compost a more homogeneous and more chemically balanced medium for the growth of the mushroom. After the mushroom mycelium develops in the manure, there is an increase in the water-soluble substances,

due to the abundance of these in the mycelium. Styer (8) also found that there is an increase in the solubility of the organic matter with the age of the mushroom bed. The nitrogen remains constant but decreases in concentration when compared with the ash content.

As to utilization of various organic substances as sources of energy and for the synthesis of the cell substances, very few investigations are available. Duggar (1) found that *Agaricus campestris* preferred starch as a source of energy rather than sugars; of the latter, maltose was most readily utilized. Proteins proved to be the most favorable nitrogen and carbon sources. The organism could also utilize inorganic sources of nitrogen, preferring ammonium salts to nitrates. Styer (9) has shown, by the use of the silica gel plate, that *Agaricus campestris* was able to attack a great variety of substances, including various sugars, especially maltose, glucose, and xylose. Cellulose was also decomposed. The growth of the organism on gum and pectin suggests the utilization of hemicelluloses by the fungus. The possibility that the mushroom can attack lignins was also indicated. Proteins were found to be excellent sources of nitrogen.

Although the evidence concerning the utilization of the various chemical constituents of the compost by the mushroom is thus found to be only fragmentary, considerable information has accumulated concerning the nutrition of Hymenomycetes which may throw some light also upon the assimilation processes of this organism. It has been shown by Falck (2), for example, that in the case of the wood destroying fungi, some organisms attack the cellulose and pentosans but not the lignins, while others are capable of decomposing the lignin as well as, and in some cases even in preference to, the cellulose, in addition of course to the pentosans and other hemicelluloses. *Merulius lacrymans* decomposes fats, pentosans and cellulose, but not lignins (Schwalbe, 7). *Coprinus radians*, however, is capable of decomposing hemicelluloses, cellulose, and lignins, the last group to a less extent than the other two (Waksman, 12).

It is important to note here that most of the bacteria, including the aerobic and anaerobic forms as well as those growing at higher temperatures, and the fungi which are active in the composting of the manure preparatory to the growth of the mushrooms, are unable to attack the lignins (12). It is also important to call attention to the extensive synthesis of microbial cell substance, as evident by the accumulation of organic nitrogenous complexes as a result of the growth of the organisms bringing about the decomposition of the cellulose and hemicelluloses in the process of composting of manure (5, 10).

Experimental Results

The following investigations were undertaken with the purpose of throwing some light upon the chemical changes taking place in the process of composting of manure preparatory to the inoculation of the mushroom

as well as the changes taking place during the actual growth of the mushroom from the spawn inoculated into the composted manure. Unfortunately the process of composting was not carried out, in these experiments, under absolutely controlled conditions and there is no assurance that the manure did not vary with the different composts from which the samples were obtained. More controlled studies are now being carried out. However, even with the samples obtained from different manure heaps, which might have varied somewhat originally in composition, the results obtained present sufficient importance to be reported here.

The first experiment deals with the chemical composition of samples of manure obtained from a series of manure heaps, of different composting periods. These samples can be designated as follows:

1, Fresh horse manure, including the straw bedding.
2, Manure from compost (12 days), one day after first turning.
3, Manure from compost (28 days), seven days after second turning.
4, Manure from compost (37 days), seven days after third turning, at filling time.
5, Compost from mushroom bed, eight days after filling (47 days old); the temperature has just fallen to normal, after the final indoor heating.
6, Compost from mushroom bed, 20 days after filling, 10 days after spawning, but spawn not included (61 days old).
7, Compost from mushroom bed, 34 days after filling, 24 days after spawning, spawn not included (71 days old).
8, Similar to 7, but filled with spawn (71 days old); no mushrooms formed as yet.
9, Spent manure, free from soil (153 days from beginning of composting).

The composite samples were brought to the laboratory, well mixed, and analyzed first for moisture and nitrogen. The samples of manure and compost were then dried at low temperatures, ground, and used for chemical analysis, according to procedure described elsewhere (14). The results of the analysis are given in tables 1 and 2, the second set of results being computed from the first on an ash free basis.

The moisture content of the manure in the composts was 64.8 to 76.4 percent. After filling the moisture began to decrease, except in Sample 7 which was taken soon after watering of the beds. The spent manure contained only 41.3 percent of moisture.

The process of decomposition can best be followed through the relative increase in ash content. In view of the fact that the mineral constituents of the manure do not volatilize as a result of decomposition but accumulate, one would expect that the increase in the mineral or ash content should run parallel with the decrease in the organic matter content. This would indicate that if the ash content increased from 9.5 percent, at the beginning

TABLE 1. *Chemical Changes Produced in Horse Manure as a Result of Composting and the Growth of Mushrooms*

Percent of Dry Material

Sample Number of Compost	Moisture Content of Fresh Material (%)	Ether Soluble	Alcohol Soluble	Water Soluble	Hemicellulose Sugar	Cellulose Sugar	Lignin	Crude Protein	Ash
1	69.6	2.6	1.9	4.6	20.0	32.5	16.7	6.0	9.5
2	76.4	2.3	1.9	4.4	13.1	29.1	20.2	8.8	17.8
3	64.8	1.7	1.4	6.6	11.5	27.8	18.9	7.7	21.4
4	69.5	1.6	1.0	5.8	10.9	26.4	20.8	9.3	22.7
5	61.3	0.9	0.9	6.7	7.2	16.2	19.1	9.8	35.9
6	54.9	0.6	1.1	6.0	4.3	9.3	18.4	9.3	50.5
7	67.4	0.6	0.8	4.5	4.5	8.1	lost	9.6	51.3
8	56.6	0.4	0.8	6.4	4.3	7.7	18.3	7.5	56.2
9	41.3	0.4	0.8	13.5	3.1	4.8	9.8	6.2	58.9

of composting or in the uncomposted manure, to 35.9 percent ash, or four times its original concentration, after 47 days of decomposition (Sample 5), that the bulk of the total organic matter has decreased to only 25 percent of the original organic matter in the fresh manure.

The figures for the protein content do not run parallel to the increase in the ash content, as one would find in ordinary composts (15). In Sample 5, it is only about two and a half times as much as in the original straw, and not four times as in the case of the ash. This is probably due to certain losses of the nitrogen as ammonia and through the reduction of nitrates to gaseous nitrogen (5) due to the rapid decomposition processes taking place during the composting.

TABLE 2. *Relative Decomposition of the Various Organic Constituents in Manure, During Composting and the Growth of Mushrooms*

On Percent Basis of Ash-free Material

No.	Ether-Soluble	Alcohol-Soluble	Hot-Water-Soluble	Hemicellulose Sugar	Cellulose Sugar	Lignin or "Lignin-humus Complex"	Crude Protein
1	2.8	2.1	5.1	22.1	35.9	18.5	6.6
2	2.8	2.3	5.3	15.9	35.4	24.6	10.7
3	2.2	1.8	8.4	14.6	35.4	24.0	9.8
4	2.1	1.3	7.5	14.1	34.1	26.9	12.0
5	1.4	1.4	10.4	11.2	25.3	29.8	15.3
6	1.2	2.2	12.1	8.7	18.8	37.2	18.8
7	1.2	1.6	9.2	9.2	16.6	49.3	19.7
8	0.9	1.8	14.6	9.8	17.6	41.8	17.1
9	0.9	1.9	32.8	7.5	11.7	23.8	15.1

The results given in table 2 permit a study of the relative decomposition of the various organic constituents in the process of composting of the manure and the utilization of specific constituents by the mushroom. Unfortunately, the lack of information concerning the loss in weight of total material does not permit us to calculate the absolute total losses of the

various organic constituents. The only way left to calculate these losses would be to assume that the mineral content of the compost remained unchanged.

The ether-soluble fraction diminished rapidly as a result of composting. In view of the fact that the greatest reduction in this fraction took place before the spawn was introduced (at time Sample 5 was taken), one must conclude that this fraction probably does not play any important role in the nutrition of the mushroom. The same is true of the alcohol-soluble fraction, which did not generally vary in any definite direction. The relative content of the water-soluble organic matter increased only very little during the composting, but a rapid increase took place with the development of mushroom mycelium, so that it amounted to nearly a third of the total organic constituents in the spent manure. This increase is largely due to the development of the mushroom mycelium, which is rich in water soluble constituents.

The four most important constituents of the organic matter of the manure are the cellulose, the hemicelluloses, the lignin, and the protein. These four groups of complexes make up 83.1 percent of the total organic constituents of the fresh manure and 58.3 percent of the spent manure. The hemicelluloses have undergone a rapid decomposition during the early stages of composting. After the spawn was introduced, the relative decrease in hemicellulose content was not as great. The decrease in the hemicellulose content of Samples 5 and 9 is only one-third of the decrease between Samples 1 and 5. This tends to indicate that the hemicelluloses of the manure do not form a very important nutrient for the growth of the mushroom.

The cellulose content presents a somewhat different picture. During the first 37 days of composting, the relative concentration of the cellulose remained practically the same. A marked change took place after the spawn was introduced. This would lead us to suspect that cellulose may play a definite role in the nutrition of the mushroom. Further information on this point is desirable.

The most interesting results are observed in the changes that took place in the relative concentration of the lignin (or "lignin-humus" complex) and protein. Up to Sample 7, there was a gradual and continuous increase in the relative concentration of these two important fractions. However, from Sample 7 to Sample 9, or during the period of active mushroom growth, both the lignin and the protein content diminished rapidly, especially the former. From decomposition studies of organic residues (10), one would expect the contrary, namely a further increase in the lignin and protein content, parallel with an increase in the ash content. The rapid reduction of the lignin and protein from Sample 7 to Sample 9 can point to only one conclusion, namely to the utilization of these complexes by the mushroom mycelium, both as sources of energy and as nutrients for the synthesis of the mycelium.

In order to compare these results with the composition of the fungus itself, an analysis is given in table 3 of the edible mushroom *Agaricus campestris*. The results show that the fungus is rich in ether-soluble and in alcohol-soluble fractions; these comprise various fats, waxes and sterols,

TABLE 3. *Chemical Composition of Agaricus campestris*

Chemical Constituents	Percent of Dry Material
Ether-soluble portion	5.14
Hot-water-soluble organic matter	42.16
Alcohol-soluble portion	7.02
Hemicelluloses	13.66
Cellulose	4.86
Lignin	0.92
Ash	13.30
Nitrogen	6.44

in which the organism seems to be particularly rich. The mushroom has also a high hot-water-soluble fraction, namely over 42 percent of the total dry material. This fraction comprises various amino acids and acid amides, fungus starch, various soluble hemicelluloses (gums) and other substances. The total hemicellulose content is fairly high, while the cellulose content is very low: even this cellulose is probably more in the nature of chitin, rather than of true cellulose, since it contained nitrogen. The ash content of the mushroom was rather high (13.30 percent) and the nitrogen content was especially high, namely 6.44 percent. By using the ordinary factor 6.25, we find that the protein content (including protein derivatives) of the fungus is over 40 percent.

It was desirable to obtain some information concerning the ability of *Agaricus campestris* to decompose the various chemical complexes of manure in pure culture. Fresh horse manure was washed thoroughly with water, well pressed to remove the excess of water and placed in flasks. These were plugged with cotton and sterilized for 2 hours in the autoclave. Four flasks were prepared. Two were inoculated with a pure culture of the organism and the other two were left uninoculated. The cultures were incubated for six weeks at 25° C. At the end of that period of time, a chemical analysis was made. The results are given in table 4.

TABLE 4. *Decomposition of Washed Horse Manure by Pure Cultures of Agaricus campestris*

Complex	Sterile Washed Manure, Control		Washed Manure Inoculated with *Agaricus campestris*	
	Relative Composition	Total Constituents	Relative Composition	Total Constituents
	Percent	Grams	Percent	Grams
Total dry matter	100	11.800	100	10.760
Total nitrogen	1.11	0.131	1.20	0.129
Cold-water-soluble organic matter	2.65	0.313	6.33	0.681
Hemicelluloses	16.08	1.899	17.71	1.906
Cellulose	28.25	3.334	26.44	2.845
Lignin complex	26.97	3.182	24.33	2.618

The organism made a good growth, but produced no fruiting bodies. The actual reduction in the organic matter was comparatively small. The organism decomposed of course larger quantities of the organic matter in the manure than shown in the analysis, but, due to the fact that it also synthesized large quantities of mycelium, the apparent reduction is comparatively small. However, certain definite facts are evident, which are in full agreement with those observed in the previous experiments. The water-soluble organic matter increased materially over the control, as a result of the growth of the fungus. It should be remembered that the manure was washed well with water before sterilization, thus reducing markedly its water-soluble constituents. The increase in these as a result of the growth of the fungus is due not so much to the actual formation of water-soluble substances in the decomposition of insoluble complexes as to the synthesis of the fungus mycelium rich in these substances, as shown in table 3.

The organism did not attack the hemicelluloses to any appreciable extent; there was an actual increase, both in the relative concentration and in the absolute amounts, due no doubt to the synthesis of the mycelium which is rich in hemicellulose. The cellulose and the lignin have undergone most marked decomposition. The loss in these two complexes can account for practically all the loss in the total manure. This loss was $11.800 - 10.760 = 1.040$ gm. The loss in cellulose was $3.334 - 2.845 = 0.489$ gm. and in lignin $3.182 - 2.618 = 0.564$ gm., the sum being $0.489 + 0.564 = 1.053$, or exactly the same as the total loss in the culture.

It should be emphasized here again that these experiments are only preliminary in nature and the data are not sufficient to draw any broad conclusions concerning the nutrition of the organism. They point, however, to new methods of attack of the problem of utilization of the various constituents in the composts for the growth of *Agaricus campestris*.

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THE ROLE OF PHOSPHORUS IN THE METABOLISM OF PLANTS

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Considering the metabolic functions of the plant in which phosphorus is at present known to play some part it seems possible that further studies of this element in its various combinations may furnish a key to disclose more of the mechanism of the life processes of higher plants. A phosphatide-carbohydrate-protein complex similar to the so-called "jecorin" of the animal liver (Maclean) is found in plants, and this would seem to carry us beyond Overton's lipoid solution theory of permeability. Phosphatides are known to be essential to autoxidation. The view is held that sugars cannot be oxidized metabolically without the intervention of phosphorus. In combinations not yet known phosphorus is held in high percentage in the composition of nucleoplasm. There is evidence also that nitrates cannot be reduced to amino acids except in the presence of an adequate supply of phosphorus.

From a survey of the literature dealing with the effects of phosphorus on vital activity, some fields of recent special study may be classified as follows:

1. The influence on alcoholic fermentation.
2. The influence on muscular contraction.
3. The phosphorus index in blood and urine as used in clinical studies.
4. Studies of phosphorus compounds in higher plants.

Our information dealing with the chemistry of plants has been developed mainly by chemists interested in nutrition, physiologists, agronomists, and students of soil fertility whose chief concern seems to have been to determine the proper economic supply of phosphorus for plant and animal nutrition. The phosphorus content of plants at different ages and stages of growth has already been well worked out in widely separated localities and on a well diversified number of plants.

MATERIALS

The plant materials used in this work were obtained from various localities, in some cases growing under widely different conditions. For example, portions of *Carnegia gigantea* were obtained from the desert Laboratory, Tucson, Arizona. For bud, stem, and leaf analyses, Norway maple was used, the material being collected just before leaf-fall in the autumn of 1929. For the sap of stem and root, silver leafed maple was used, obtained in the spring of 1930. Somewhat later, wild grape growing on the campus was also used for the last mentioned purpose. Leaves and

young growth from box elder growing near the laboratory were selected to determine the presence of hexose phosphates (in June, 1930).

Portions of pumpkin, bean, and tomato plants were taken from a farm garden near Madison late in the summer of 1929. Tomato, potato, and sweet potato plants were grown in the laboratory greenhouse during the winter of 1929–30. Seeds of corn and of wheat were secured from a uniform supply; the corn was a yellow dent variety and the wheat was high grade Marquis.

Methods of Analysis

The microchemical method for the determination of phosphorus found best adapted to this work is known as the Bell-Doisy (1920) method as modified by Briggs (1922), and also somewhat improved by later workers. It has its origin in the Neumann (1902) wet-ash method which has been made a colorimetric one by Taylor and Miller (1914) who discovered that many phenol-reducing agents reduce ammonium phosphomolybdate in the presence of excess molybdic acid. Bell and Doisy selected hydroquinone and found that colors could be developed better by the addition of sodium sulfite. Briggs improved the method by eliminating the use of Na_2CO_3, thus eliminating greenish hues which often interfere with the determination. More recent improvements of this method include heating the preparation on a water bath for 15 minutes so that color is developed more quickly and is more stable. Another improvement offered by Roe, Irish and Boyd (1926) consists in hastening the wet oxidation of tissue by the use of H_2O_2 (supraoxid). Thus improved this method is found to be more convenient and fully as accurate as the so-called ceruleo-molybdate method of Deninge so widely used in the analysis of soils and of plant tissues.

As applied to the analyses herein given 0.1 gm. of dry matter or 1.0 gm. of fresh tissue is ashed with 1.5 cc. of an acid mixture (H_2SO_4, 7 : HNO_3, 3) in a large test tube (20 by 200 mm.). Eighteen to twenty-four test tubes can be prepared at once and set under a hood on an electric hot plate at a temperature of 120° C. When the contents of the tubes have become brown they are cleared by the addition of H_2O_2 drop by drop. The process of clearing is repeated from time to time until the contents of the tubes remain clear. They are then cooled and transferred with water to 50 cc. volumetric flasks. Before raising to volume, 2 cc. of ammonium molybdate solution (25 gm. ammonium molybdate dissolved in 300 cc. H_2O + 200 cc. of a solution consisting of 125 cc. H_2O + 75 cc. H_2SO_4) and 1 cc. of 0.5% hydroquione in 20% Na_2SO_3 solution are added. Raised to volume the flasks are set on a water bath for 15 minutes, cooled in running water and read on the colorimeter against standards varying not more than 10 mm. in reading from the unknown. The calculation is made easily by the use of the following formula.

$$\frac{\text{Standard reading}}{\text{Unknown reading}} \times \text{mg P in Standard} = \text{Amount of P in sample.}$$

Phosphorus fractions can be obtained only approximately from the tissues of plants by extraction. The lipoids are completely removed after 24 hours extraction with alcohol-ether (3 : 1) at 37–50° C. Lipase and glycerophosphatase are inactivated by these solvents. The solvents must be removed and renewed quite frequently, at least five or six times, during the period. The liquid is then decanted into a second test tube and evaporated to dryness by placing the tube in an inclined position on a sand-bath at 65° C. The residue may then be digested and analyzed by the Bell-Doisy method. The residue from the lipoid extraction is dried to remove alcohol and ether. To inactivate phytase and other phosphatases the dried material is covered with boiling water and left on a water bath to boil for 15 minutes. Theoretically this should result in obtaining only the inorganic fraction, but actually one gets approximately one-third of the total phosphorus present in the tissue.

It is generally stated by those who use the Collison method (extraction with acidic alcohol and precipitation with NH_4OH) that the inorganic phosphorus in plants is low, seldom more than ten percent of the total extract, but the Collison method is not adapted to micro-methods. We should call the fraction secured by water extraction simply water soluble phosphorus. When the plant tissue contains large quantities of pentosans and mucilaginous material, as in cactus tissue, the extraction must continue longer, often for one week, with frequent hot water changes. The decanted extract is kept evaporating until completely dry, when it may be digested and determinations made.

The residue from the water extract is next extracted with 1–1.25% HCl in watery solution. Phytin, the hexose phosphates, and starch-bound phosphorus are presumably removed after twenty-four hours of this acid extraction if the temperature is kept rather high (35°–50°) and the extract occasionally renewed. This extract when digested and determined gives a fair estimate of the carbohydrate and inosite-bound organic phosphorus.

The more resistant nuclein remains as a final fraction in the residue from previous extractions. This nuclein phosphorus may be measured by the Bell-Doisy procedure after complete digestion of the remaining residue.

The hexose phosphates, such as have been found in yeast and in seedlings of higher plants, have been separately determined by Robison (6) and by Neuberg and Leibowitz (7). The method of Robison separates hexose monophosphates from hexose diphosphate on the principle that both these phosphates are precipitated by barium in an alcoholic solution. The dried precipitate yields its hexose monophosphate as a barium salt soluble in cold water. The hexose-diphosphate is then removed by extraction from the remaining barium salts. These fractions are purified by precipitation with lead acetate, the lead being removed by hydrogen sulphid and the latter by aeration. To obtain a sufficient quantity to secure molecular weights Robison cultivated yeast in glucose phosphate solutions. No practical

means has been found to increase the normal hexose phosphates in the juices of the higher green plants. To obtain data as to the presence or absence of hexose phosphates in the juices of green plants, Robison's process, above described, was carried out until clear solutions were obtained, which were then tested colorimetrically for phosphorus and microchemically by the Shaffer-Hartman (1921) micro-method for sugars. The results indicate the presence of hexose monophosphate. There is some evidence of hexose diphosphate in the juices of tomato, of potato, of wheat, and of corn seedlings but the evidence was not so conclusive as for hexose monophosphate, hence it is not offered.

Experimental Results

Determinations of Total Phosphorus

An examination of the analytical data in the literature on the phosphorus content of the various plant organs furnishes a suggestion as to the relative importance of phosphorus in the metabolism of such organs. In general, meristematic tissue, actively synthetic tissue, and fruiting organs are found to be rich in phosphorus as compared with transport or supporting tissues. Storage organs are rich in phosphorus only in parts where organic phosphorus-containing compounds are accumulated.

An excellent example illustrating distribution of phosphorus in vegetative tissue has been found in the tree cactus (*Carnegia*). Portions of a plant about 40 feet high were available in the form of cross sections representing base, one-third the distance from base to apex, two-thirds, and the top portion. The sections were 10–14 inches in depth and 14–18 inches in diameter. The normal living tissue of each section was separated as pith, inner cortex (inner one-half of white cortex lying next the xylem cylinder), middle cortex (white cortex lying between the inner half and the outside chlorophyllous portion), outer cortex approximately one centimeter deep consisting of the chlorophyllous and pigmented periphery with the cuticle removed. These separate portions were finely ground in a food chopper, weighed and rapidly dried. After taking dry weights the tissue was ground to a fine powder for sampling. In table 1 the phosphorus determinations on this material may be compared with those of nitrogen and potassium in the same material.

In this plant where tissues are massive enough to supply large quantities of homogeneous tissue for representative sampling the trend of increase or decrease is very significant. It will be seen that nitrogen and phosphorus increase from center to periphery, but that potassium presents a much more abrupt curve in the opposite direction. From bottom to top potassium follows the same trend of increase as phosphorus and nitrogen. If we assume that the periphery is metabolically more active than the pith and the top more than the base then phosphorus and nitrogen are directly

TABLE 1. *Results of Analyses of Cactus (Carnegia), mg. per 100 Grams Dry Matter*

Part	Percentage of Dry Matter	Total Amount of Phosphorus in Milligrams	Total Amount of Nitrogen in Milligrams	Total Amount of Potassium in Milligrams
Base pith	18.34	33.04	230.00	354.38
Base inner cortex	20.31	29.70	585.00	11.02
Base mid cortex	18.18	28.00	737.50	8.80
Base outer cortex	19.08	55.00	1355.00	6.55
* L/3 pith	11.48	5.68	300.00	2153.37
* L/3 inner cortex	7.85	37.95	577.50	194.54
* L/3 mid cortex	8.54	42.19	692.50	72.60
* L/3 outer cortex	11.90	60.36	1647.50	74.51
† U/3 pith	7.35	7.57	477.50	4862.68
† U/3 inner cortex	5.88	46.19	852.50	1108.53
† U/3 mid cortex	7.00	38.76	842.50	502.08
† U/3 outer cortex	10.78	85.47	2015.00	151.36
Top pith (meristem)	4.48	142.01	1590.00	6043.36
Top inner cortex	4.23	127.55	1602.50	5570.36
Top mid cortex	5.07	105.26	1677.50	2058.15
Top outer cortex	8.52	133.33	2445.00	352.25

* L/3 = lower one-third section. † U/3 = upper two-thirds section.

TABLE 2. *Comparison of Various Plants and Organs, mg. per 100 Grams Dry Matter*

Part	Percentage of Dry Matter	Total Phosphorus in Milligrams
Cactus (*Carnegia*)		
Tree growing tip	8.52	142.01
Cactus (*Carnegia*), entire plant (Age 8–10 months)	5.36	492.65
Norway Maple		
Winter tip buds	24.68	661.50
Leaves near turning	51.50	332.57
Tomato		
Growing tips	12.65	844.60
Young leaves	12.65	790.50
Bean vine, growing tips	14.86	727.45
Pumpkin vine		
Growing tip	11.74	1482.11
* Leaf blade	20.94	382.48
Leaf petiole	7.93	454.46
Germinating wheat		
Shoots	10.14	1314.89
Roots	11.17	813.87
Corn plant		
Leaves	24.05	302.42
Nodes	11.52	275.00
Internodes	11.52	246.12
Roots	8.89	166.64
Sweet potato		
Tuber (cortex)	38.23	162.71
Tuber (stele)	38.56	126.45
Young plants (entire)	24.00	240.00

* *Note.* Allowance must be made in tissues in which silicious secretions in cuticle or other parts raises the percentage of dry matter. The true figure representing phosphorus in metabolic tissue is thereby unduly lowered.

related to rate of metabolism while potassium apparently has functions more indirectly related to it.

In this slowly growing plant the percentages of the essential elements (Ca excepted) are low as compared with more rapidly growing plants. If our conclusion is correct as regards phosphorus in relation to metabolism, analyses of more rapidly growing plants should show higher percentages of phosphorus in direct proportion to the activity of the tissue. Such analyses have been carried out for a variety of tissues. The results are displayed in table 2.

That such a list is only relatively indicative is shown clearly by leaves of pumpkin and of corn, and this is attributable to variable amounts of such substances as cutin which are not concerned in metabolic processes but which necessarily enter into the calculations. Arendt's (1859) comment on his analysis of the oat plant illustrates his early recognition of this fact. "The quantity of minerals used by plants fluctuates with the chemical and physical nature of the soil, the genus and character of the plant, the age of the plant, and the nature of the plant organs using the mineral."

In the further study of this problem the tomato plant was used as a means of determining in what parts or organs the greatest amounts of phosphorus exist at different ages and stages in the development of the plant. The results (table 3) will be found to check closely with those of McGillivray (1927). Stems are found to be consistently low in total phosphorus; the roots are slightly higher; meristematic tips and immature leaves are only slightly higher than actively synthesizing leaves, both having approximately the maximum amount of phosphorus present in vegetative organs. The highest phosphorus content is found in flowers. Except for No. I, all plants here analyzed were grown during the winter season in the greenhouse. Growth was slow, light being a limiting factor.

On casual observation it might seem that floral parts obtain their high phosphorus content from the stamens, but when ratios are worked out on the basis of dry matter, remembering the proportion of non-metabolic substance in each tissue, it is seen that differences in phosphorus content between active vegetative tissue and floral parts or flowers as a whole are not great.

Organic and Inorganic Phosphorus Fractions

Lipoids. Of the four groups of phosphorus compounds in plants the lipoids are easiest to separate. Table 5 indicates that lipoids are most abundant in the most metabolically active tissues. Young tubers, however, seem to have essentially the same content as roots. Lipoids are probably not connected with starch formation. Opinion is divided in the literature regarding increase of lipoids during germination. In this instance it was diminished and apparently changed to other forms.

Water Soluble Phosphorus. This group contains not only the inorganic phosphoric salts but quantities of hexose phosphates that are sufficiently

TABLE 3. *Total Phosphorus Determinations on Tomato Plant at Different Ages Shown in Milligrams of Phosphorus per 100 Grams Dry Matter*

	Description	Percentage of Dry Matter	Total Phosphorus in Milligrams
I.	Summer growth. Fruiting plants in open field		
	Growing tips	12.8	844.60
	Leaf blades	12.8	781.25
II.	Tomato plants from greenhouse. Seeded Oct. 4, 1929. Age of plants 5 weeks		
	Tops, stem and leaves	6.35	544.22
	Roots	6.04	382.77
III.	Nine-week plants		
	Tops, including stem and all leaves	7.63	497.22
	Stems below 1st leaves	6.00	147.06
	Roots	6.66	298.50
IV.	18-week plants		
	Tops and immature leaves	13.51	741.20
	1st fully expanded leaf below top	10.12	655.72
	2d fully expanded leaf below top	8.02	512.80
	3d fully expanded leaf below top	7.93	382.80
	4th fully expanded leaf below top	7.59	328.82
	5th fully expanded leaf below top	8.04	301.83
	6th fully exapnded leaf below top	8.20	291.26
	7th fully expanded leaf below top	9.27	292.20
	8th and all old leaves	11.82	305.07
	Upper 1/2 stems	4.54	258.06
	Lower 1/2 stems	6.94	160.64
	Roots	10.00	213.90
V.	22-week plants		
	Tops and immature leaves	11.67	446.92
	Flowers	13.00	540.54
	Upper 1/2 leaves	9.30	375.00
	Lower 1/2 leaves	9.84	274.44
	Upper 1/2 stems	5.88	262.87
	Lower 1/2 stems	7.40	163.13
	Roots	13.58	201.26
VI.	Tomato, old plant		
	Tops	12.72	493.80
	Upper 1/2 leaves	11.77	233.11
	Lower 1/2 leaves	12.68	121.95
	Upper stem	10.59	131.58
	Lower stem	12.68	119.11
	Root	13.55	307.68
	Green fruit	6.98	297.36
	Ripe fruit	11.07	246.12

labile to break down in the slight acidity of the tissue in water even after care has been taken to eliminate enzymatic action. That soluble phosphorus is found in greatest quantity in growing tips, young tubers, and germinating beans might be taken as an index to greater lability in such tissues.

TABLE 4. *Total Phosphorus in Floral Parts Compared with Other Tissue*

Material	Percentage of Dry Matter	Total Phosphorus in Milligrams	Ratios		
			Grams	Dry Matter	Milligrams of Phosphorus
Pumpkin					
Leaf blade	20.94	382.48	1	18	1/4
Vine tip	11.74	1482.11	1	126	1/4
Corolla	6.17	1364.56	1	221	1/5
Stigma	7.97	1034.75	1	130	
Ovary	7.97	1262.60	1	171	
Stamens	8.00	2267.50	1	283	1/2
Corn					
Leaf blade	24.06	302.42	1	12	3/5
Ear (time of fertilization)	8.46	1125.73	1	133	
Tomato					
Leaf blade	9.30	375.00	1	40	
Growing tip	11.67	446.92	1	39	
Flowers	13.00	540.54	1	41	
Green fruit	6.98	297.36	1	41	
Ripe fruit	11.07	246.12	1	22	
Hibiscus (Abutilon Darwiniana)					
Leaves	36.00	206.44	1	5	3/4
Petals	13.30	291.96	1	22	
Stamens	47.65	517.24	1	11	
Lily					
Anthers	35.54	644.88	1	18	

Acid Soluble Phosphorus. This group includes starch, phytin, and hexose phosphates not dissolved or hydrolysed by water.

Nuclein. In table 5 the nuclein phosphorus is shown to be higher in some instances than might be expected. No explanation is offered unless the previous extractions were incomplete.

In this connection it is of interest to note that most if not all starches contain phosphorus, probably bound as a carbohydrate ester. This bound phosphorus varies in amount according to the kind of starch and the quantity of amylopectins present. In some starches phosphorus is partially

TABLE 5. *Organic and Inorganic Phosphorus Fractions*

Milligrams per 100 Grams Dry Matter

Plant Organ	Lipoidal Phosphorus	Water Soluble Phosphorus	Acid Soluble Phosphorus	Nuclein Phosphorus
Sweet potato				
Growing tips	95.32	250.00	119.40	25.47
Active leaves	100.00	126.98	111.00	13.33
Leaf petioles	14.68	102.56	125.00	28.57
Roots	45.64	82.47	35.55	34.20
Young tubers, pencil size	47.24	200.00	34.80	10.64
Soy Bean				
Dry seed	112.67	95.23	52.27	74.76
6 days germinated	40.40	145.45	100.00	58.82
Cactus (*Carnegia*)				
Top outer cortex	4.61	11.84	12.85	3.55

replaced by fatty acids. A composite table by Samec (1913, 1914) on the phosphorus content of different starches, and by Taylor and Nelson (1920) on the fatty acids of starch has been compiled by Karrer (1925) and is quoted in table 6. I have also appended further tests on different starches and related substances. Samec states that fatty acids never completely replace phosphorus but Pieser (1926) reports instances in which she finds starch containing no phosphorus. It would seem significant that substances closely related to starch, such as maltose, inulin, raffinose, cellulose, and gum arabic contain no phosphorus.

TABLE 6. *The Phosphorus Content of Starch and Kindred Substances*

Kind of Starch	P_2O_5	Fatty Acids
Potato from Karrer's (13) data	0.112%	nil
Meadow saffron from Karrer's (13) data	0.016	
Arrow-root from Karrer's (13) data	0.031	
Cassava from Karrer's (13) data	0.012	0.12%
Horse- hestnut from Karrer's (13) data	0.016	0.56
Cucuma from Karrer's (13) data	0.162	
Wheat from Karrer's (13) data	0.105	
Maize from Karrer's (13) data	0.034	0.61
Rice from Karrer's (13) data	0.039	0.83
Glycogen from Karrer's (13) data	0.072	
Tests in This Laboratory		
Scarlet runner bean	0.104%	No tests
Potato (laboratory supply)	0.106	
Tapioca (commercial supply)	0.016	
Corn starch (commercial supply)	0.029	
Rice starch (commercial supply)	0.058	
Soft wheat starch (commercial supply)	0.131	
Hard wheat starch (commercial supply)	0.136	
Dextrin (chemical supply)	0.006	
Maltose (chemical supply)	No phos.	
Inulin (chemical supply)	No phos.	
Raffinose (chemical supply)	No phos.	
Cotton (absorbent)	No phos.	
Gum arabic	No phos.	

EVIDENCE FOR THE PRESENCE OF HEXOSE PHOSPHATES IN HIGHER PLANTS

After the discovery by Harden and Young (1908) of the true relation between phosphorus and sugars in alcoholic fermentation Young (1911) proved the product to be hexose diphosphoric acid. Later Robison (1922) isolated a hexose monophosphate from yeast juice. Robison, and Neuberg and Leibowitz (1927) have separately shown that a part of the hexose phosphates of alcoholic fermentation caused by yeast is always hexose monophosphoric acid while the major portion is hexose diphosphoric acid. In animal physiology particularly during muscular contraction hexose monophosphoric acid is most abundant. In germinating seeds hexose monophosphate has been reported by Barensheen and Albers (1928).

The phosphorus content of starch is thought to be attached as a hexose phosphate (Samec, 1913). Meyerhoff (1924) states that the initial step in bringing about a labile condition of sugar for its breakdown and the subsequent release of energy in alcoholic fermentation and in respiration in yeast and in muscle is accomplished by the formation of hexose phosphates. If this be true of yeast and animal respiratory processes, it would probably be true in the higher green plants; yet nowhere in the literature do we find proof. Barensheen and Albers (1928) have reported a gain in organic acid soluble phosphorus in *Elodea canadensis* at time intervals in the light following a food reducing period in darkness.

To test for the presence of hexose phosphates in the higher green plants the following experiments were tried with results as shown. 500 cc. of tomato juice was pressed from ten pot-grown plants. Robison's method as above described was used. After three precipitations with lead acetate, 225 cc. presumably of hexose monophosphate solution and 200 cc. of hexose diphosphate solution were obtained. Five-cc. samples of these solutions were tested by the Shaffer Hartmann micromethod. The average yield of glucose per sample was 0.477 milligrams, a total for the original 500 cc. of juice of 21.465 mg. If this were in hexose monophosphate form, by calculation we should have $\frac{21.465}{180} \times 31 = 3.69$ milligrams of phosphorus. By the Bell-Doisy test of this solution 3.8 mg. of phosphorus were found. The hexose diphosphoric acid solution gave no appreciable reduction of copper oxid and only a trace of phosphorus was evident. Hence there was probably little or no hexose diphosphoric acid present in this juice of the tomato plant.

To test further for the presence of hexose phosphates in the active tissues of some higher plants, 1 kg. of fresh leaves and shoots of the box elder, collected June 15, 1930, was ground in a food chopper and placed in a six percent solution of trichloracetic acid. This tissue was passed through a hydraulic press and the extract treated as above described for the juice of the tomato. The results are similar; 15.086 mg. of glucose were found. By calculation as hexose monophosphate this would call for 2.6 mg. of phosphorus. Three milligrams of phosphorus were found. No hexose-diphosphate could be found. This is in accord with Barrenscheen and Albers (1928) who found only hexose monophosphate in wheat and rye seedlings six days after germination.

The above evidence strongly indicates the presence of hexose monophosphate in the juices of green plants. Therefore it is of interest to compare the reactions of plant juices and their contained enzyms with the reactions of muscle juices as done by Embden, Meyerhoff, and their followers. Corn and wheat seedlings six days after germination were frozen. The endosperms were removed from the corn but in the case of wheat the intact sprouted grain was used. This material was ground in a food chopper

and enough distilled water was added to release the frozen juices under hydraulic pressure. The extract was then returned to the freezing point until all could be used at once.

The juices were then exposed to a temperature of 25° C. Each one was mixed thoroughly by pouring, divided into four parts, and set to aerate at a room temperature of 23° C. One sample was used for immediate analysis, followed at 3, 6, and 9-hour intervals by the remaining fractions. The method of analysis was that of Robison as above described. Results are shown in table 7. The process was not carried beyond clearing from the first lead precipitate and doubtless both phosphorus and sugars other than those bound as hexose phosphates were present in the filtrates, which would account for the lack of balance between the phosphorus and glucose for the respective hexose phosphates. This is very evident in the case of the wheat extract which included endosperms as well as young seedlings. The significant thing is in the reduced quantity of both phosphorus and sugar in each consecutive test.

TABLE 7. *Corn Seedling Extract in Enzymatic Change*

Treatment	Sample	Monophosphate Filtrate		Diphosphate Filtrate	
		Total Phosphorus in Milligrams	Total Glucose in Milligrams	Total Phosphorus in Milligrams	Total Glucose in Milligrams
Immediate analysis	250 cc.	2.25	7.58	5.58	56.68
After aëration 3 hrs.	250 cc.	2.21	7.51	2.68	37.13
After aëration 6 hrs.	250 cc.	1.95	7.41	2.05	43.04
After aëration 9 hrs.	250 cc.	1.43	6.44	1.90	28.52
Wheat Seedling Extract in Enzymatic Change					
Immediate analysis	750 cc.	12.00	67.83	12.77	416.62
After aëration 3 hrs.	750 cc.	7.58	98.02	7.44	392.60
After aëration 6 hrs.	750 cc.	8.07	91.01	6.74	341.26
After aëration 9 hrs.	700 cc.	7.36	65.36	4.82	356.65

Seedlings are different from other growing plants, which are not dependent upon high food reserves. Therefore an attempt was made to follow both anabolic and catabolic systems in plants not dependent upon stored food reserves. For this purpose potato plants grown in flats were used. These plants were past prime with many etiolated leaves. They were kept 36 hours in darkness, then all plants were brought into the light at the same time. One sample was analyzed at once, the others after 3, 7, and 10-hour exposures to daylight. The day exposure however was not one of full sunlight but the light was interrupted by cloudy periods. The results are set down in table 8. In this experiment both phosphorus and sugar increase in sunlight. This is in accord with Barrenscheen and Albers' (1928) findings with *Elodea canadensis*.

TABLE 8. *Analyses of Potato Plants for Hexose Phosphates after Periods of Light Exposure*

Potato Plants Without Roots	Crude Barium Precipitate, Dry Weight in Grams	Hexose Monophosphate Filtrate		Hexose Diphosphate Filtrate	
		Total Milligrams of Phosphate	Total Milligrams of Glucose	Total Milligrams of Phosphate	Total Milligrams of Glucose
After 36 hrs. in darkness..	12.6	4.94	0.0	4.58	5.57
After 36 hrs. in darkness + 3 hrs. in light.....	13.3	6.35	7.61	5.00	7.96
After 36 hrs. in darkness + 7 hrs. in light.....	9.7	4.75	12.61	3.91	10.53
36 hrs. in darkness + 10 hrs. in daylight.....	12.6	7.19	11.36	5.76	15.12

Efforts were made to obtain some insight into the catabolic system of tomato plants. The method of treatment was the same as above described but the process of exposure to light and darkness was reversed and the time between analyses extended to twelve-hour periods. Table 9 shows the results and contributes further evidence that hexose phosphates are present in the higher green plants and that at least one of their functions is concerned with respiration.

TABLE 9. *Analyses of Tomato Plants after Exposure to Periods of Darkness for Hexose Phosphates*

Tomato Plants Without Roots	Crude Barium Precipitate, Dry Weight in Grams	Hexose Monophosphate Filtrate		Hexose Diphosphate Filtrate	
		Total Milligrams of Phosphate	Total Milligrams of Glucose	Total Milligrams of Phosphate	Total Milligrams of Glucose
After 14 hrs. daylight.....	20.0	9.61	39.53	5.71	10.92
After 12 hrs. darkness	17.8	6.80	25.48	4.05	7.33
After 24 hrs. darkness	12.0	6.41	18.35	4.46	3.59
After 36 hrs. darkness	15.3	6.53	19.09	3.29	2.74

DISCUSSION

In the light of certain biochemical studies on plants during the past fifteen years phosphorus appears to have five main functions in the metabolism of the green plant. 1. In the roots according to Eckerson (1929) the function of nitrate reduction may be ascribed to phosphorus. 2. In leaves and other green parts phosphorus has a relationship to the synthesis of starch. 3. Phosphorus is connected in some way with respiratory processes. 4. Phosphorus functions in nuclear division. 5. In cytoplasm as a constituent of lipoids phosphorus is held by some to contribute to the selective absorption by control of surface charges (Levene, 1921) or by influencing the selective solubility of cytoplasmic membranes.

The function of phosphorus in nitrate reduction has not been considered in this study nor its relationship to nuclear activity. The functions of

lipoids in absorption are still in the form of hypotheses based on the properties of diamino-monophosphatides according to Grafe and Magistris (1926). These authors find a part of these phosphatides to be water soluble and another fraction "inactivated" is soluble only in organic solvents. In combination these are said to form a colloidal-phosphatide-protein cell membrane to which lability is contributed by the phosphatide modicum. Stewart (1928), after following Grafe's methods, refutes their conclusions. Nevertheless these hypothetical opinions must stand until new evidence is brought forth.

The present study is chiefly concerned in the relation of phosphorus to the release of energy. The results of Harden and his co-workers in their studies on yeast and of Embden's school on the metabolic phenomena of muscle juice indicate a strong probability that phosphorus performs similar functions in the higher green plants. Few attempts have been made to carry over this idea to green plants. Only one, that of Barrenscheen and Albers (1928), appears in the literature so far as I am able to find. If hexose phosphates are to be found in green plants, it is probable they may hold the same relationship to fermentation and respiration in these higher plants as they do in fermentation by yeast or in muscle contraction. Meyerhoff and Lohmann (1927) state that the general idea among biochemists is that in the fermentation of sugars intermediary esters are formed in a nascent state but perhaps not in the hexose phosphate forms isolated by chemical means. Gottschalk (1927), studying the action of a co-zymase in connection with hexose phosphates, concludes that fermentation caused by yeast results in a splitting off from "glycogen" small fractions of alpha, beta, or balanced glucose which is phosphorylated perhaps by the aid of co-zymase by the action of which is liberated a readily decomposable form of sugar which is susceptible to seizure by destructive enzyms.

Leclerc du Sablon was the first to suggest that fats are easily changed to sugar in the cells of plants. Kostychev (1927) probably voices the opinion of most plant physiologists when he says "Respiration of fat is associated with a low value of CO_2/O_2 and undoubtedly is effected by way of the intermediate stage of sugar." Animal physiologists, however, are less positive, asserting that such changes from fat to sugar have never been proven.

Setting controversy aside, it is certain that by whatever mechanism proteins and fats are oxidized the major portion of respiratory energy in both plants and animals is derived from fermentable sugars. It must be admitted also that some change takes place as a first step which renders these sugars unstable. Such lability would seem to be acquired in some manner by phosphorylation, and phosphorylation according to Gottschalk is accomplished by the heat stable coferment, the activator of zymase, identical with the lactic acid coferment of milk and muscle, a phosphorylation activator or cophosphatase. Once activated a complex of enzyms carry the process through anaerobic respiration and finally through aerobic respiration.

Animal physiologists have been able to extend biochemical knowledge of respiration of muscle farther than plant physiologists have carried knowledge of respiration in plants. Regarding respiration in plants Kostychev says, "Quantities of oxidizing and fermenting enzyms are so coordinated in the plant cell that the velocity of the primary sugar cleavage is less than the velocity of the subsequent oxidation so that alcohol is never formed when air is admitted." The Zaleskis (1929) also report that alcohol formed in seed germination has never been found to be resynthesized. In muscular contraction, however, where lactic acid is the product of the first cleavage, approximately four-fifths of the acid produced by the anaerobic process is known to be resynthesized with the phosphorus radical as hexose monophosphate. Recently Blackman (1928) has advanced an hypothesis of respiration in plants in order to explain an observed difference in rate of glycolysis over carbon dioxid elimination in respiring apples. The ratio of glycolysis to carbon dioxid elimination he finds is 3 or 3.5 to 1. This product of glycolization which is amenable to final oxidation was not determined as to character nor traced back to the original catabolyte, as has been done in muscle juice. Since the product does not accumulate, it is assumed to be built back in a similar manner as is the resynthesis of lactic acid in animal tissue.

Before the principles which underlie the process of respiration can be accepted with finality they must be found to apply perhaps with modifications to all forms of life. Until recently, however, this study has not been more than superficially carried to the higher green plants. Buds and germinating seeds have been shown (Zaleski and Mordkin, 1928) to exhibit phenomena parallel with fermentation caused by yeast. Barrenscheen and Albers (1928) have recently reported having followed quantitatively hexose phosphoric esters in *Elodea canadensis* and in seedlings of wheat and rye. *Elodea canadensis* seems to have been the only representative of green plants to be subjected to examination for variation in hexose phosphates during respiration. By extending the research on this subject to the tomato, the potato, and the box elder the present study adds to the evidence that the higher green plants are not different in their initial respiratory steps from other forms of life. In all forms of life thus far examined phosphorus seems to stand on the threshold between potential and kinetic energy relations.

Summary

1. From data obtained for total phosphorus content in widely different tissues and organs of plants it seems evident that the greatest concentration of phosphorus in any plant is present in the region of greatest metabolic activity.

2. Phosphorus content in plants is relative and subject to environmental and hereditary factors.

3. True comparative total phosphorus content in tissues calculated on

a dry matter basis must take into account the relative amounts of non-metabolically active tissue such as cutin, fiber, etc. which in variable quantities accompanies the true metabolically active tissue.

4. Carbohydrate phosphate esters apparently serve the same purpose in the higher green plants as they do in muscular contraction and in fermentation caused by yeast.

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EFFECT OF SOME INORGANIC AND ORGANIC MERCURIALS ON GROWTH OF *LUPINUS ALBUS*

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The work of the author on the effects of drugs and poisons on living plants and living plant protoplasm (Macht, 1930) has been found useful from the pharmacological point of view, since it offered a convenient method of comparing the toxicity of various drugs and chemicals, on the one hand, and the relative toxicity of different concentrations of individual drugs or chemicals, on the other. In the present investigation a study was made of the effects of solutions of a number of organic and inorganic mercury compounds on the growth of seedlings of *Lupinus albus*. This is of value not only from the purely scientific point of view but also practically for the detection of inorganic mercurial adulterants added to solutions of pure organic mercury compounds.

METHODS

Seedlings of *Lupinus albus*, large variety, were used in these experiments because of their almost absolute uniformity of germination and hardihood for the purposes of the problem. The seeds were soaked in tap water for twenty-four hours and germinated in finely ground moist sphagnum moss kept at room temperature in the dark. When the seedlings had attained a root length of from 30 mm. to 45 mm., they were ready for use. Several rows of hard-glass test tubes were set up in perpendicular position on a specially constructed board, each row consisting of eight or ten tubes. These were filled with a mixture of water and physiological nutrient solution (Shive, 1915). The solution employed in this investigation contained 10.4 cc. of a 0.5 molar solution of calcium nitrate, 30 cc. of a 0.5 molar solution of magnesium sulfate, and 36 cc. of a 0.5 molar solution of mono-potassium phosphate with sufficient distilled water to make a liter.. In most of the experiments performed by the author, such a Shive solution is mixed with an equal part of distilled or tap water, as the case may be, for physiological experiments. A much weaker mixture of Shive medium and water had to be used with some of the mercurials because the hydrogen-ion concentration of the half-and-half mixture was too acid for some of the compounds studied and caused their precipitation. This was particularly true of mercurochrome-220 soluble, which is an alkaline salt, and is precipitated as an insoluble yellow compound in an acid medium. It was found that when one-half or even one percent of Shive solution was mixed with water, the resultant hydrogen-ion was not acid enough to produce such precipitation of mercurochrome.

In these experiments, one row of seedlings was used as a control and a series of other rows were made up to contain different solutions of the mercurials to be examined. Seedlings of approximately the same length were carefully selected, rinsed in water, and measured in respect to the length of their roots. They were then placed in the various solutions to be examined, and the whole was put away in the dark for twenty-four hours. At the end of that time the increment in the length of the roots was measured and the growth in the various solutions was compared with that of the normal controls and expressed in form of percentages, which the author named the *phytotoxic index.*

Results

The following mercurial compounds were studied: mercuric chlorid or bichlorid of mercury, which is freely soluble in water; mercuric iodid or "red iodid" of mercury, which is practically insoluble in water but can be readily dissolved in water by the addition of sodium iodid; mercuric nitrate, of which traces could be dissolved in the mixture of Shive solution and water employed in the experiments; mercury bisulfate, of which minute traces could also be dissolved in the same way; oxi-mercuri-di-brom fluorescein or mercurochrome-220 soluble; di-mercuri mercurochrome or a mercurochrome containing two atoms of mercury instead of one; mono-mercuri fluorescein or flumerin; mono-hydroxy-mercuri-di-iodo-resorcin phthalein or merodicein, a new mercurial recently described by Dunning and Farinholt (1929); nitro-anhydro-hydroxy-mercuri-ortho cresol; and sodium-ethyl-mercuri-thio salicylate. The growth of seedlings of *Lupinus albus* in a number of concentrations of these mercury solutions was studied by the method described above at the temperature of 22° C. in the dark. The results obtained are exhibited in table 1. It will be noted that all the

TABLE 1. *Comparative Toxicity of Some Organic and Inorganic Mercurials for Lupinus albus Seedlings at Temperatures of 22° C.*

Compound	Concentration	Phytotoxic Index
Oxy-mercuri-di-brom fluorescein (mercurochrome)	1 : 5,000	25%
	1 : 10,000	34%
	1 : 20,000	40%
Mono-hydroxy-mercuri-di-iodo-resorcin phthalein (merodicein)	1 : 5,000	28%
	1 : 10,000	70%
	1 : 20,000	80%
Di-mercuri mercurochrome	1 : 20,000	45%
Mono-mercuri fluorescein (flumerin)	1 : 20,000	51%
Nitro-anhydro-hydroxy-mercuri-ortho cresol	1 : 10,000	0%
	1 : 20,000	0%
Sodium-ethyl-mercuri-thio salicylate	1 : 10,000	0%
	1 : 20,000	0%
Mercuric chlorid (bichlorid of mercury)	1 : 20,000	0%
	1 : 40,000	0%
Mercuric iodid	1 : 20,000	0%
	1 : 40,000	0%
Mercuric nitrate	trace	0%
Mercury bisulfate	trace	0%

inorganic mercury compounds were very toxic for the plants. In a short time they produced plasmolysis and death. Most of the organic compounds, on the other hand, were found to be much less toxic, and this was particularly the case with mercurochrome and merodicein. The mercuric iodid was used in a solution of sodium iodid but its toxicity was not due to the admixture of the latter. Control experiments with sodium iodid alone showed that it was not toxic at all when used in a solution of 1 : 1,000 and even stronger. It is well known that when mercuric iodid is dissolved with sodium and potassium iodid a double salt is formed. We must therefore conclude that the toxic effects on the plants are produced by this double salt as such. On the other hand, mercuric chlorid exhibits the characteristic effect of a protoplasmic poison which is ascribed to mercuric ion; and this must also be the case with the weak solutions of mercuric nitrate and mercury bisulfate.

In view of the extensive medicinal use of oxi-mercuri-di-brom fluorescein or mercurochrome, a more elaborate study was made of this organic mercurial, the results of which are exhibited in table 2. Growth of *Lupinus*

TABLE 2. *Effect of Various Concentrations of Mercurochrome*

Concentration of Mercurochrome	At Temperature of 25° C.	At Temperature of 19° C.
1 : 1,000	19	0
1 : 2,000	24	0
1 : 3,000	27	0
1 : 4,000	30	18
1 : 5,000	35	23
1 : 10,000	40	33
1 : 20,000	46	40
1 : 30,000	50	46
1 : 40,000	55	52
1 : 50,000	57	58
1 : 100,000	60	62
1 : 200,000	62	71
1 : 300,000	65	83
1 : 400,000	71	90
1 : 500,000	78	100
1 : 1,000,000	94	108
1 : 2,000,000	104	115

albus seedlings in various concentrations of mercurochrome, from 1 : 1,000 to 1 : 2,000,000, at two temperatures, 19° C. and 25° C., or room temperature, was studied. As may be seen from the table, the results are very interesting. At the lower temperature it was found that solutions from 1 : 1,000 to 1 : 4,000 killed the seedlings. Weaker solutions produced a distinct inhibition in proportion to concentration of the drug. At room temperature definite inhibition was also exerted by the mercurochrome solutions, an inhibition which was also proportional to the concentration used. In both sets of experiments, of course, when extremely weak dilutions

were employed, a limit was reached where no inhibition could be ascertained. This is in agreement with the results found in studying various concentrations of all kinds of drugs and temperatures not only on plants but animals as well.

Discussion

It is interesting to note the marked difference between organic compounds, on the one hand, and most of the inorganic compounds, on the other. The author's findings, from the pharmacological point of view, serve to emphasize the organic nature of such compounds as mercurochrome, merodicein, flumerin, etc. These chemicals contain mercury in firm organic combination and when brought in solution are not dissociated but exert their pharmacological effects by virtue of the molecule as a whole. It is well known that the toxicity of the inorganic mercurial compounds is largely due to free play of the mercury ions. The effects of the various solutions on the growth of the seedlings were not due to excessive changes in the hydrogen-ion concentration as control experiments indicated.

The author has taken advantage of the observations above in regard to the marked difference in toxicity between inorganic mercurial compounds and mercurochrome for developing a method of detecting adulterants in mercurochrome solutions. As the result of many experiments, he has found that the action of even small traces of mercuric chlorid and of other inorganic mercury compounds, added to solutions of pure mercurochrome, markedly increase the toxicity of such solutions. Indeed, the presence of small quantities of even metallic mercury in mercurochrome solutions can be detected by the phytotoxic test because an appreciable amount of the metallic mercury will be dissolved by the physiological solution and produce a definite increase in the toxicity of the contaminated mercurochrome as compared with a control solution of the absolutely chemically pure oxi-mercuri-di-brom fluorescein.

All the experiments performed in the present investigation were made on the growth of *Lupinus albus* seedlings in various solutions in the dark. The author has also investigated the effect of light on the toxicity of the various mercurial compounds and more particularly of the mercurial dyes. It is well known that the fluorescent dyes are pharmacologically often more active in the presence of dye than in its absence. The author has shown this to be true for mercurochrome in connection with various pharmacological tests (Macht, 1928). In working with the *Lupinus* seedlings, it was found that sunlight rendered mercurochrome solutions and also those of merodicein somewhat more toxic for the *Lupinus* seedlings, and when the experiments were made with ultraviolet rays, this toxicity was even more marked. The results of these experiments are reserved for another paper.

SUMMARY

1. A number of inorganic and organic mercurial compounds in solution were studied on the growth of seedlings of *Lupinus albus*.

2. A marked difference in toxicity was noted between the inorganic mercury and most of the organic mercury compounds studied.

3. The toxicity of various concentrations of oxi-di-brom fluorescein at 19° C. and 25° C., respectively, was studied on a more elaborate scale.

4. As a result of these phytotoxic studies, a method has been developed of detecting the presence of inorganic mercurial impurities or adulterants which have been added to solutions of oxi-mercuri-di-brom fluorescein or mercurochrome.

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ON THE ALLEGED STIMULATING ACTION OF X-RAYS UPON PLANTS

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Contradictory results are reported by those who are experimenting with the effects of light doses of X-rays upon plants. Practically all who have investigated medium to heavy doses agree that these cause a checking action on growth. A majority of those experimenting with light doses, however, report a stimulating action, while others believe that if there is an apparent stimulation, it is but an acceleration of growth following a checking which may occur immediately after exposure.

The word "stimulation" in this paper is used in its more popular sense to indicate a positive reaction. If an acceleration of the growth rate were produced by X-rays, this would be referred to as a stimulating effect of the rays. A statement that light dosages stimulate growth would mean that an acceleration of the growth rate was produced which resulted in an increase of dry weight.

A more technical meaning of the term indicates merely the response to a stimulus, whether positive or negative. Using the word in this sense, one would speak of any response to the rays as a stimulation even though a retardation of growth or destructive action should result. It is possible that some of the confusion arising in the literature on the effects of light doses of X-rays upon plants and animals may be due to these two interpretations of the term. The writer believes that the usage of the term as indicating increased or quickened activity is so general that one hearing of a stimulating effect associates it with the idea of increased cellular growth. It is this interpretation of the word which one should keep in mind while considering the results given in the present paper.

Miege and Coupe (7) write of the favorable influence of X-rays upon underground portions of the radish and peppergrass. Halberstaedter and Simons (2) report that medium doses give stimulating effects to the growth of bean and wheat seedlings. Altman, Rochlin, and Gleichgewicht (1) and Maldiney and Thouvenin (6) conclude that germination is stimulated by

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X-rays. Koernicke (5) states that light doses favor the development of the plant and the activity of the processes which normally take place. Rivera (11) writes of finding doses which stimulate development of the aërial portions of the Windsor bean. Reiss (10) reports that if radiation is not too strong, buds of *Syringa* are forced by X-rays. Patten and Wigoder (8) say that exceedingly small doses given to *Vicia Faba* cause more rapid growth.

Data are here presented to support the conclusion that the stimulative influence which X-rays are supposed to exercise on plants does not exist. When large numbers of plants are treated and a large number of controls are grown under identical conditions of culture, plants irradiated with weak doses either show decreased growth or behave like the controls. They do not show stimulation.

Effect of Light Dosage upon Leaf and Stem Development of Seedlings

Seedlings of tomato, sunberry, and sunflower were irradiated with very light doses of X-rays which have been reported to cause increased growth. These seedlings were grown in the University greenhouse, irradiated, and kept growing on the same bench with controls of the same age, alternating with them so that all environmental conditions were practically the same except for the irradiation. At the conclusion of each experiment, the tops were cut off at the ground line, quickly weighed for the determination of the green weight, and the absolute dry weight then determined.

Solanaceae. Tomatoes have proved to be rather more resistant to heavy doses of X-rays than some other species. Series of experiments were conducted to determine whether young seedlings of the Solanaceae could be stimulated to greater growth by exposure to light dosage. In series I the young seedlings of tomato and sunberry were given one exposure. They were allowed to grow for 25 days to allow full time for the appearance of any stimulating influence that might be present. Previous experiments (4) have established the fact that the effects of irradiation tend to disappear toward the end of the third week after irradiation if a second dose is not given.

In table 1 are given results which show in but one instance an increase in weight of irradiated plants of Series I over the control. In this case the increase is very slight. Since one weak dose caused no stimulation, two doses were given to both tomato and sunberry seedlings. In one case the tops were taken for determination of weight at the end of 6 days, in the other case the seedlings were allowed to grow for about three weeks before being cut.

In the case of both tomato and sunberry seedlings, neither one nor two exposures to light irradiation caused an increased growth over that of the control.

TABLE 1. *Growth of Seedlings of Solanaceae after Exposure to Weak Irradiation*

Series I, Plants given one exposure (84 K.V., 5 ma., distance 30 cm. for 1 min.)

Name of Plant	Age of Seedlings when Irradiated	Days after Irradiation when Tops Were Taken	No. of Plants		Average Green Weight (in mg.)		Average Dry Weight (in mg.)	
			Control	Irradiated	Control	Irradiated	Control	Irradiated
Tomato...	16	25	30	33	3030	2570	330	263
Sunberry..	11	28	13	16	440	300	46	30
Sunberry..	22	30	16	11	1870	2000	247	243
Sunberry..	22	48	12	77	4600	4500	624	511

Series II, Plant given two exposures; first as above, second, 84 K.V., 5 ma., 45 sec.

	Dose								
	I	II							
Tomato...	16	29	6	16	15	5530	4650	620	480
Tomato...	16	29	24	16	16	8141	6668	1130	931
Sunberry..	11	17	6	10	10	597	516	57	53
Sunberry..	11	17	19	12	13	2608	2650	247	235

Sunflower Seedlings. Sunflowers are quite sensitive to X-rays, therefore this plant should show stimulation with light doses if any plants do One hundred and ten 7-day-old seedlings of practically the same size were placed into two groups. One group was given one exposure to weak dosage. Measurements from the base of the stem to the growing point were taken after 5, 8, and 15 days. Results are shown in table 2. One group of plants was taken for dry weight determinations 8 days after irradiation and a second group 15 days afterwards. Results show that weak irradiation has not stimulated growth.

TABLE 2. *Growth of 7-day-old Sunflower Seedlings Exposed to Weak X-radiation (120 K.V., 5 ma., 1 min., distance 30 cm.)*

Days after Irradiation	Height of Stem				Absolute Dry Weight of Tops			
	Control		Irradiated		Control		Irradiated	
	No. of Plants	Av. Ht. (cm.)	No. of Plants	Av. Ht. (cm.)	No. of Plants	Av. Wt. (mg.)	No. of Plants	Av. Wt. (mg.)
0	56	2.32	54	2.42				
5	54	6.52	55	6.58				
8	54	7.96	55	7.74	34	119.86	34	119.77
15	20	13.90	19	13.08	20	239.57	19	219.07

Results show that there is no more difference in growth between the control and treated plants than there would be between two groups of controls. At no time during the 15 days of growth did there occur an increase in

height which could be interpreted as stimulation. The absolute dry weight of the tops of the irradiated plants was slightly less than that of the controls both in the group taken at the end of 8 days and in the group which grew for 15 days.

Effect of Light Doses upon Growth of Entire Plant

Vicia sativa. The dose used on *Vicia sativa* seedlings was that reported by Patten and Wigoder (8) as having caused increased growth of *Vicia Faba* seedlings. The seedlings of group *A* were grown in sphagnum and the experiment was concluded 7 days after irradiation. Table 3 shows that the amount of growth measured by the absolute dry weight was less in the irradiated seedlings than in the controls. Seedlings of group *B* were grown in silt for 15 days after irradiation. Silt is easily washed from roots, hence the entire root growth could be determined with very little error. The average green weight and average absolute dry weight as well are both less in the irradiated seedlings than in the controls.

Table 3. *Growth of Vicia sativa Seedlings after Exposure to Weak Irradiation (120 K.V., 5 ma., 45 sec., Distance 30 cm.). Seedlings of A Were Grown in Sphagnum; Seedlings of B, in Silt*

Age of Seedlings when Irradiated	Days after Irradiation when Weight Was Taken	Weight of Entire Plant*					
		No. of Plants		Average Green Weight (mg.)		Average Dry Weight (mg.)	
		Control	Irradiated	Control	Irradiated	Control	Irradiated
A. 94 hr.....	7	282	267	291	299	46.8	41.8
B. 7 days....	15	267	274	603	544	61.7	55.0

* Weights given for *B* are for the entire plant, exclusive of seed.

A very large number of seedlings was used in this experiment and no appreciable difference between growth of control and irradiated seedlings was shown when the absolute dry weights were taken as a measure of growth.

Vicia Faba. Forty-hour seedlings of *Vicia Faba* were irradiated with a dosage of 120 K.V., 5 ma. current, 30 cm. distance, for 45 seconds,—a dose which Patten and Wigoder (8) state produces more rapid growth in seedlings of this species. The time selected for irradiation was between 9 and 10 A.M. as Reinhard and Tucker (9) found this to be one period when *Vicia Faba* root tips were most sensitive.

After 10 days' growth in sand, the seedlings were carefully dug and adhering sand particles were rinsed off. Measurements were made of lengths of roots and stems. Both green and absolute dry weights of stems and roots were determined. Tabulated results given in table 4 indicate that in no case did the irradiation cause a stimulation of growth.

TABLE 4. *Growth of Vicia Faba Seedlings Ten Days after Exposure to Light Irradiation (120 K.V., 5 ma., 1 min., dis. 30 cm.). Results Are for 77 Controls and 79 Irradiated Seedlings*

	Av. Wt. of Sprouted Seed (gm.)	Roots			Stems		
		Length (cm.)	Av. Green Wt. (gm.)	Av. Dry Wt. (gm.)	Length (cm.)	Av. Green Wt. (gm.)	Av. Dry Wt. (gm.)
Control.....	4.28	4.45	.256	.0208	3.96	.411	.040
Irrad.....	4.27	3.63	.189	.0159	3.44	.346	.032

A sufficiently large number of *Vicia Faba* seedlings was used to indicate that when either length of radicle, or green weight or dry weight of the roots and stems was used as a measure of growth, the above dose of X-rays does not cause increased growth.

EFFECT ON TUBERS AND BULBS

Tulip Bulbs, I. Bulbs of two varieties of tulips were separated into two groups so that those which were to serve as controls were of practically the same size and weight as the lot to be treated. Bulbs of one group of each variety were irradiated with a light dose and planted in a garden plot early in November. The control bulbs were planted in the same row as the irradiated ones, alternating with them.

TABLE 5. *Vegetative and Floral Development of Tulips Given One Light Dose of X-radiation. Variety Recorded as A is Pride of Haarlem; B, Baronne de la Tonnaye*

Floral Development (Results Are Averages from 19–23 Plants)				Vegetative Development (Results Are Averages from 22–25 Plants)									
				Leaf Development				Bulb Development					
Length of Mature Peduncle (cm.)		Maximum Expanse of Petals (cm.)		Basal Leaf Length after 184 Days (cm.)		Second Leaf Length after 205 Days (cm.)		Av. No. Bulbs per Plant		Av. Wt. of Bulbs per Plant (gm.)		Av. Wt. of Bulbs (gm.)	
Cont.	Irrad.	Cont.	Irrad.	Cont.	Irrad.	Cont.	Irrad.	Cont.	Irrad.	Cont.	Irrad.	Cont.	Irrad.
*A.** 30.56	29.28	12.70	12.73	23.10	23.60	16.13	16.52	5.04	5.68	27.49	28.97	5.45	5.09
B.† 28.79	28.23	13.27	12.90	21.20	22.08	18.97	19.60	2.27	2.58	27.32	27.50	12.02	10.64
Gain in yield from irradiated bulbs over controls (percentages)													
A.‡ −4.1		.23		2.1		2.4		12.5		5.3		−6.4	
B. 1.9		−2.7		4.1		3.3		13.6		.65		−11.4	

* Dose for *A*, 7½ inch spark gap, 7 ma., 1 mm. Al filter, dis. 19 inches for 60 sec.

† Dose for *B*, 5 inch spark gap, 5 ma., no filter, 19 inches for 69 sec.

‡ The minus sign indicates that the irradiated is less than the control.

The measurements given in table 5 were made to determine whether there had been any stimulation in vegetative growth or floral development.

The bulbs were left in the soil until they were mature as was indicated by the tops which had died down. After the bulbs were dug they were spread out in a thin layer in an airy room and dried for two days. Any adhering particles of dirt were wiped off before they were weighed. Tabulated data show that there is but little variation in vegetative growth, or size of blossoms. There is, however, an increase in the number of bulbs produced by plants from irradiated material over those produced by the controls.

Irradiation of bulbs with one light dose does not seem to affect materially the leaf and flower production. In general, the leaves were slightly longer in the plants whose bulbs had been treated. The average number of bulbs produced by experimental plants was 13 percent greater than those produced by the controls. Before considering this evidence as conclusive proof of stimulation of bulb development, it was decided to grow tulips a second year in greater numbers and note particularly whether the bulb development was increased.

TABLE 6. *Leaf and Bulb Development of Four Varieties of Tulips. Before Planting, the Bulbs Were Treated with One Light Dose of X-radiation*

	Sieraad Van Flora		Pride of Haarlem		White Queen		Baronne de la Tonnaye		Total from 4 varieties	
	Cont.	Irrad.	Cont.	Irrad.	Cont.	Irrad.	Cont.	Irrad.	Cont.	Irrad.
No. of plants measured..	10	11	12	11	16	17	15	14	53	53
Av. height of each plant (in cm.).....	25.6	26.13	25.2	25	34.8	36.1	34.5	33.5	30.8	31
No. of plants measured..	8	10	8	9	16	17	15	14	47	50
Av. length of each peduncle (in cm.).....	26.62	27.7	29.8	29.1	30.2	26.8	31.3	31.03	28.6	28.5
No. of plants forming bulbs.....	13	13	15	16	16	15	15	14	59	58
Av. number of bulbs per plant.....	3.92	3.61	3.06	3.5	4.31	5.06	3.4	2.92	3.67	3.79
Av. weight of bulbs per plant (in gm.).....	25.12	27.3	23.92	28.31	33.02	32.97	33.51	31.45	29.09	30.05
Av. weight of bulbs (in gm.).....	6.40	7.55	7.80	6.40	7.65	6.5	9.85	10.74	7.91	7.92

Gain or loss in yield from irradiated bulbs over controls (percentages)

Height from base to tip of leaf.....	2.07	−.79*	3.75	−3.06	.6
Av. length of mature peduncle.....	4.13	−2.35	−11.25	−.95	−.3
Av. number of bulbs per plant.....	−7.90	14.37	17.40	−4.11	3.2
Av. weight of bulbs per plant.....	8.67	18.06	−.15	−6.14	3.4
Av. weight of bulbs.....	17.96	−17.94	−15.05	9.0	.12

* The minus sign indicates that the irradiated is less than the control.

Tulip Bulbs, II. The procedure followed was similar to that given in the previous experiment with tulips. The dose used had the following set-

up: $7\frac{1}{2}$ inch spark gap, 5 ma. current, distance $48\frac{3}{4}$ cm., filter 1 mm. Al. for 1 minute. The bulbs were planted in a trench 6–8 inches in width in which sand had been placed. After the ground was completely frozen, the beds were covered with fertilizer to hold the frost in. This keeps the bulbs dormant and prevents too early spring growth. When the bulbs were dug in mid-summer, they were all allowed to dry the same length of time before weighings were made.

Table 6 shows the measurements of height, peduncle length, and bulb development of four varieties of tulips for a total of 59 control and 58 experimental plants. By comparing the averages from all varieties it may be noted that the average height, the average length of peduncle, and the average weight of bulbs is practically the same for both controls and irradiated material. The average number of bulbs from irradiated material is but 3.2 percent greater than for the controls while the average weight of bulbs per plant is 3.4 percent greater than for the controls. It is most likely that there would be this amount of variation between two sets of controls.

The data presented in tables 5 and 6 giving results from tulips grown for two successive years indicate that these bulbs were not stimulated to more vigorous flower production nor to greater bulb development by the use of X-rays.

Colorado Wild Potato, I. If weak irradiation causes no stimulation of bulb development, it is reasonable to suppose that tuber growth will not be affected by it. Jacobson (3) reports that by irradiation the crop of one variety of potatoes was increased 84 percent in weight over the control plants while with another variety the increase was as much as 200 percent. He states that each tuber was larger than normal and that there was also an increase in total number of tubers.

Preliminary experiments with greenhouse-grown cultivated potatoes (*Solanum tuberosum*) indicated that irradiated mother-tubers produced more potatoes to the hill than did the controls and that the average weight per hill from the irradiated seed-tuber was 4.69 percent more than the control.

The Colorado wild potato (*Solanum Jamesii*) was selected for experiments as only a very limited number of the cultivated potatoes could be grown in the greenhouse at one time. The wild potato is well adapted to greenhouse cultivation for it matures rather quickly and does not require as much room for growth as do the cultivated potatoes. The native range of this plant is from the mountains of Colorado to New Mexico and Arizona. In Colorado it is found in the extreme southwestern part of the state. The whole plant averages from eight to ten inches in height in nature. Under cultivation, it grows somewhat taller. The leaves are pinnate and the 7–9 leaflets lanceolate to ovate-oblong. The blossom is white and has a corolla which is more star-shaped than the rotate corolla of the cultivated potato. The tubers range in length from 6 to 30 millimeters; their average weight is less than a gram. Those which were obtained from Arizona were light russet in color and showed prominent lenticels.

The potatoes were sorted so that those which were to serve as controls were of practically the same size and weight as the lot to be treated. Immediately after irradiation, the tubers were planted in two plots in the University greenhouse. Controls in an equal number were planted in the same rows with the irradiated tubers, alternating with them in both directions. Some irradiated tubers as well as controls were planted in 5- and 6-inch pots. All tubers were exposed to practically the same experimental conditions except for the irradiation. One portion of the experimental group received dosage amounting to 420 ma. sec. with a spark gap of $7\frac{1}{2}$ inches; the second lot received 900 ma. sec. with a spark gap of 6 inches. Those of the second group were exposed for a slightly longer time but the rays were softer because of the lower voltage.

The crop was harvested by hills when the vines were dead. Three to four inches was the usual depth at which the tubers were found. It was necessary to sift the soil of the plots in order that the very small tubers should not be lost. The tubers from each hill were rubbed free of any adhering particles of soil, counted, and weighed by hills. Table 7 shows results from the two dosages with totals and averages from all hills.

TABLE 7. *Effect of X-rays upon Tuber Development of the Colorado Wild Potato*

	Lot *A*		Lot *B*		Results from Both Lots	
Dosage	420 ma. sec. with $7\frac{1}{2}$ in. spark gap		900 ma. sec. with 5 in. spark gap			
	Control	Irrad.	Control	Irrad.	Control	Irrad.
No. of hills	62	61	48	48	110	109
No. of tubers	484	506	666	713	1150	1219
Total wt. in gm.	415.3	494.4	505.4	533.9	920.7	1028.3
Av. wt. per tuber	.858	.977	.758	.748	.80	.843
Av. no. per hill	7.80	8.29	13.80	14.80	10.45	11.18
Av. wt. per hill	6.70	8.10	10.50	11.10	8.37	9.43

Results shown in percentages:

Av. wt. per tuber of irradiated is 5.37 percent more than control
Av. no. per hill of irradiated is 6.98 percent more than control
Av. wt. per hill of irradiated is 12.66 percent more than control

These results indicate that there is some slight stimulation in crop production when light doses of X-rays are applied to the tubers of wild potato.

A record of the time of sprouting for the plants in the plots is shown in table 8. Controls sprouted in from 26 to 42 days with the average length of time 32.3 days. Sprouts from irradiated tubers appeared above ground in slightly less time. Records for time of blossoming showed that for both controls and irradiated material the average length of time before blossoms appeared was the same.

TABLE 8. *Sprouting and Blossoming of Irradiated and Control Plants*

	Days (after Planting) Before Sprouts Appeared above Ground		Days (after Planting) Before Blossoms Appeared	
	Control	Irradiated	Control	Irradiated
Lot *A*	31.8	30.3	57	58
Lot *B*	32.59	31.0	56.9	57.1
Av. for both lots	32.3	30.7	57.0	57.4

Colorado Wild Potato, II. Over 14,000 tubers were grown this past year in three different habitats. In each case the tubers were selected in lots of equal weights. Each experimental group was irradiated with dosage from the following set-up: 84 K.V., 7 ma. current, for 1 minute. A 1 mm. Al filter was used. One group of the irradiated tubers with an equal number of controls was planted in the University greenhouse in a bed especially prepared to provide sandy loam soil 13 inches in depth.

TABLE 9. *Yield of Irradiated and Untreated Colorado Wild Potatoes Grown in Various Habitats*

	Altitude of 8,000 ft.		Altitude of 6,000 ft.		Greenhouse		Total from all Habitats	
	Control	Irradiated*	Control	Irradiated	Control	Irradiated	Control	Irradiated
No. of hills	92	91	43	41	84	83	219	215
Total no. of tubers	3029	3072	2715	2425	1473	1318	7217	6815
Total wt. of tubers (in gm.)	2396.3	2386.6	2541.3	2236.3	934.4	982.5	5872	5605.4
Av. wt. per tuber (in gm.)	.791	.776	.936	.922	.634	.745	.813	.822
Av. no. per hill	32.92	33.75	63.14	59.1	17.53	15.75	32.95	31.69
Av. wt. per hill (in gm.)	26.04	26.22	59.1	54.5	11.12	11.83	26.81	26.07

Gain or loss in yield from irradiated tubers over controls (percentages)

	Altitude of 8,000 ft.	Altitude of 6,000 ft.	Greenhouse	Results from yield in three habitats
Av. wt. per tuber (in gm.)	17.51	−1.495†	−1.89	1.10
Av. no. per hill	−10.15	−6.39	2.54	−3.82
Av. wt. per hill (in gm.)	6.38	−7.78	.691	−2.76

* Dosage given to all treated tubers: 84 K.V., 7 ma. current, 30 cm. distance, 1 mm. Al filter. Exposure for 45 seconds for those grown at an altitude of 6,000 feet. 1 min. exposure for other groups.

† A minus sign indicates the irradiated is less than the control.

The second group of tubers was planted in the lower foothill region at Boulder, Colorado, where the altitude is about 6000 feet. The soil of the plot was a mellow loam. The third group was planted in a plot at the head of Boulder Canon at an altitude of 8000 feet. The area where the tubers were planted was in a region of freshly plowed sod. The soil for the most part was loam but some regions contained sand washed from a road near-by.

Table 9 shows the yield from the three plots. Plants grown at an altitude of 8000 feet produced more than twice as many tubers per hill as did those grown in the University greenhouse. The yield from the garden plot at 6000 feet was more than three and one half times as many per hill as for those grown in the greenhouse. The average weight of tubers produced at 6000 feet was 35 percent greater than for those grown in the greenhouse while those at 8000 feet were 28 percent heavier.

Results from table 9 indicate that very light doses of X-rays cause practically no increase in the yield of the Colorado wild potato. Although the average weight per tuber from irradiated seed-tubers was slightly more, the average number per hill and the average weight per hill was less in those from the treated tubers than in the controls.

Records were kept of the time of the appearance of the sprout above ground and the time of blossoming for those grown in the greenhouse. Table 10 shows that while shoots from the irradiated tubers did appear above ground in slightly less time than did the controls, still the difference is hardly great enough to be significant. Plants from the irradiated tubers blossomed at practically the same time as did those from the untreated tubers.

TABLE 10. *Sprouting and Blossoming of Wild Potatoes in Greenhouse*

Days after Planting before Sprouts Appeared above Ground						Days after Planting before Blossoms Appeared					
Dormant Tubers Planted March 29											
Control			Irradiated			Control			Irradiated		
No.	Total	Av.	No.	Total	Av.	No.	Total	Av.	No.	Total	Av.
71	3536	49.8	74	3453	46.6	57	4284	75.1	59	4429	75
Sprouted Tubers Planted May 27											
No.	Total	Av.	No.	Total	Av.	No.	Total	Av.	No.	Total	Av.
46	563	12.2	46	518	11.2	42	1524	36.2	41	1455	25.5

Plants grown for two successive years in the greenhouse agree in showing that there was a slight reduction in time of the appearance of sprouts from irradiated tubers over that required for the controls. Time of blossoming

was practically the same for plants from the irradiated and untreated tubers in the groups of plants grown for two successive years in the greenhouse.

Discussion

Statements appearing in the literature on the subject of the influence of light exposures of X-rays upon plant tissues are contradictory, for many report stimulation with such exposures while others deny it. Results obtained by observing the growth of seven species of plants fail to show any indication of increased growth by experimental plants over the controls.

Seedlings of tomato, sunberry, sunflower, and two species of vetch were given one and in some cases two weak doses of irradiation. After the plants had grown for periods of sufficient length for the detection of any stimulative action, comparisons were made of green and dry weights of experimental and control plants. In two groups, root growth was considered as well as growth of tops and in two groups, stem height was considered also. In all cases studied, a considerable number of treated plants with a like number of controls were grown under the same environmental conditions. It is believed that those who have reported stimulation with weak doses did not use a sufficiently large number of specimens and used other means of judging amount of growth than measurements of height and determinations of green and dry weights.

The first series of experiments with the effect of weak irradiation on development of underground stems indicated that there might be some increased growth of these parts by weak irradiation. The second year, twice the number of varieties of tulips were planted and results clearly indicated that no more variation in leaf, floral, or bulb development was present between specimens from experimental plants and the controls than between two sets of plants from untreated bulbs.

Studies made on the Colorado wild potato, *Solanum Jamesii*, yielded data which show conclusive evidence of non-stimulation. The tubers were grown for two successive years and the results for the second year were drawn from over 14,000 tubers grown at different altitudes. While the average weight per tuber from irradiated seed-tubers was one percent greater, the average number per hill and the average weight per hill was less in those from the treated tubers than in the controls.

If there exists stimulation of plant tissues by light doses of X-rays, it seems most questionable whether the exact dosage for plants has yet been discovered. The doses reported as stimulative do not regularly cause increment of growth in leaf, floral, or underground parts in any of the species studied.

Summary

Numerous seedlings of tomato, sunberry, sunflower, and two species of vetch were irradiated with light X-ray dosages which have been reported to cause stimulation. No increased growth of the experimental plants over

the controls was evidenced by measurements of height and green and dry weight determinations.

Tulip bulbs and Colorado wild potato tubers grown from irradiated material showed some indication of increase over the controls in the preliminary experiments. On growing these plants a second year, however, in greatly increased numbers, it was apparent that the X-rays had caused no stimulation, for the controls and experimental plants did not vary more in yield than would have two groups of untreated plants.

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TWO NEW CHYTRIDIACEOUS FUNGI FROM COLD SPRING HARBOR

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During the course of an examination of the Phycomycete flora of the region around Cold Spring Harbor, Long Island, two very interesting members of the Cladochytriaceae were encountered. Inasmuch as members of this group are of rather uncommon occurrence, and, since the two forms in question appear to be hitherto undescribed species, the preparation of this brief account has seemed advisable.

Cladochytrium Nowakowskii n. sp.

This extremely virulent parasite was found in a well balanced algal culture which contained *Spirogyra crassa* Kütz., two undetermined species of *Oedogonium*, young thalli of *Coleochaete*, and several other members of the Chlorophyceae, collected in 1928 in the old Fish Hatchery Pond. The fungus was at once detected in the infected cells because of its extensive polyphagous mycelium which was characterized by the presence of numerous septate turbinate cells (Pl. XLV, *N*). Further, wherever sporangia were being produced, the parasite was easily discovered by reason of the brilliant orange oil globule possessed by each of the zoöspores. Free zoöspores were also abundant in the surrounding medium.

As non-sexual reproduction was occurring in abundance and infection of the algal cells by the zoöspores was being accomplished with great frequency, the life history of the organism could be followed in many cases on all three of the algal genera mentioned in the preceding paragraph.

The zoöspore, coming to rest upon a host cell, generally moves for a time in an amoeboid fashion over the surface. Finally, it becomes quiescent, assumes a spherical shape and apparently absorbs its cilium. The hitherto colorless protoplasm is now a pale yellow. There is then produced an exceedingly tenuous, hyaline tube, which penetrates the cell wall of the host and through which the content of the encysted zoöspore (cystospore), its oil globule intact, passes into the alga (fig. *I*). This movement is a slow and deliberate one and, after its occurrence, the wall of the empty cystospore may be seen adherent to the outer wall of the host. Once inside of the alga, the protoplasm of the fungus assumes a nearly spherical form, somewhat larger than the mass of the original zoöspore. From this fungous protoplasm, after several minutes, a few delicate hyphae grow out in all directions and soon establish the vegetative phase of the parasite (fig. *J*). The oil globule disassociates and the protoplasm which had originally es-

tablished the infection seems to be used up in the formation of the mycelium. The latter ramifies profusely throughout the host filament, its branches generally arising in a dichotomous manner.

At frequent intervals along the mycelium, narrow, fusiform swellings appear, which gradually enlarge to rounded-oblong, often nearly spherical, turbinate cells. The strongly vacuolate protoplasm of these cells is divided into two equal parts by a transverse septum (fig. *N*). These structures vary from 13–15.6 μ in length by 5.2–10.4 μ in width, while the filamentous portion of the mycelium is usually exceedingly tenuous, never attaining a diameter of more than 1.5 μ.

It is not known with certainty whether or not the sporangia may originate, as Nowakowski pointed out in the case of *Cladochytrium tenue* (5), from the turbinate cells. Certainly, some of the latter structures become markedly spherical, the septum is obliterated, and the cell increases in size. Whatever its early origin, the immature sporangium is nearly spherical to ovoid, and as it matures, increases in size, apparently at the expense of its concomitant hyphae and turbinate cells which are drained of their protoplasm. The latter fact would seem to indicate that the septum of the turbinate cell does not completely divide the two parts. When the sporangium attains a diameter of about 15 μ (13–18 μ), its content, until now a dull gray, becomes tinged a faint yellow. As maturation continues, the color, contained in minute droplets, increases in its intensity and becomes a brilliant orange. The droplets now become organized into definite globules about 4 μ in diameter, which are regularly placed in the colorless, finely granular, protoplasm. During the ensuing cleavage of the zoöspores, there is produced, usually opposite the point of attachment of the hypha, a discharge tube which penetrates the wall of the host and protrudes about 2 μ out into the water. The diameter of this tube, which is highly refractive at its apex, is about 1.5–2 μ. As has been intimated, coincident with the formation of the discharge tube, the protoplasm of the sporangium is blocked out into 5–15 polygonal portions, the spore initials, each with a centrally disposed, orange oil globule.

Zoöspore emergence is initiated by a barely perceptible movement of the spore initial nearest the tube. Generally the flow of the protoplasm is observed before motion can be detected in the oil globule, the more obvious portion of the spore. Once started, the zoöspores flow successively, in a slow, steady fashion, through the discharge tube, each oil globule being necessarily much constricted during this process, but emerging intact with its attendant protoplasm. Outside, at the mouth of the discharge tube, the zoöspores form a motionless, compact, spherical mass, surrounded by an almost invisible vesicle (fig. *H*). The latter structure is apparently formed from the highly modified, refractive apex of the tube. No cilia could be observed as the spores emerged, although the extreme tenuousness of the former structures might easily have rendered them invisible against the

homogeneous background of the sporangium. After remaining at the mouth of the discharge tube for 5–10 minutes, the vesicle breaks and the spores, thus released, gradually start to lash their cilia, now quite visible, and dart away.

The zoöspore, when swimming, is nearly spherical to discoid in shape, about 7.3 μ in diameter, with an orange oil globule located toward the anterior part of the body (fig. *M*). Often the globule appears to protrude from the body of the spore. The single cilium, possessed by each spore, is about 6–7 times the diameter of the spore in length and is attached by a very fine line of granules to an ovoid structure, probably the nucleus, which lies midway between the periphery of the oil globule and the point of emergence of the cilium. The darting, forward motion of the zoöspore is accompanied by a twirling movement. At times the spore may suddenly come to rest on an algal cell and move in a distinctly amoeboid fashion. During these intervals, the oil globule may lose its spherical shape and conform to that of the body of the spore (fig. *M*). It was noticed that whenever amoeboid motion took place, the protoplasm of the spore became excessively vacuolate. The spore may suddenly resume its swimming and dart away to another substratum, perhaps to come to rest permanently, and to penetrate the host cell. Germination may also occur when the spore is not in contact with an algal cell, in which case, after the retraction of the cilium, there is produced a tenuous hypha which branches in a dichotomous fashion and may form one or more fusiform swellings. Growth, however, is decidedly limited under these conditions and no instances of such a mycelium penetrating a host cell were observed.

The sporangia proliferate profusely, in some instances as many as six being formed successively within the walls of the original one. The "secondary" sporangium originates by a protruding of the apex of the attached stalk into the old sporangial cavity. This sporangium increases in size, usually nearly filling the old wall, and produces a discharge tube which, however, does not always grow through the old tube but generally bores through the wall, forming a new exit through the host cell wall to the exterior (fig. *K*).

No sexual reproduction has been observed to occur in this fungus. However, a type of resting spore was found in one instance. This structure was intramatrical, spherical, about 14.4 μ in diameter, and possessed a somewhat thickened wall. Further, the tenuous outer wall which surrounded this spore had collapsed in a characteristic manner around the latter structure. The content was densely granular, colorless, and possessed a centrally disposed orange globule about 7 μ in diameter (fig. *L*). The whole structure was attached to the typical vegetative thallus by a somewhat tapering stalk. Although the method of formation of this body is not known, it would seem by reason of its hyphal connections to belong to the fungus under discussion. This point cannot be settled with certainty, however, until more material is available for study.

The fungus was cultured for a time on bacto-cornmeal agar. It seemed to have no difficulty in maintaining an existence on this medium, but due to the slow rate of growth, it was impossible to free it from bacteria. The mycelium, in culture, was characterized by the formation of an abundance of septate turbinate cells.

The Cold Spring Harbor organism is obviously closely related to *Cladochytrium tenue* Now., which is generally considered the only valid species of the genus (von Minden, 4; Fitzpatrick, 2). From a perusal of the literature it is evident that generic concepts in this group of the chytrids are in need of clarification. A more detailed taxonomic consideration of this and other forms will be included in the writer's paper on the Phycomycetes of the Cold Spring Harbor region. It seems sufficient for the present to point out the differences between *C. tenue* and this new species, *C. Nowakowskii*.[1] The possession of so conspicuous a feature as the orange pigmentation of the oil globule of the zoöspore immediately segregates our fungus from *C. tenue*. Further, the diameters of the sporangia of *C. Nowakowskii* are consistently smaller than those of Nowakowski's fungus (13–18 μ cf. 18–66 μ), although the zoöspores of the latter organism are smaller (5 μ cf. 7.5 μ). These two differences, consequently, give rise to another constant difference between the two, namely, the number of zoöspores produced in a sporangium. In *C. tenue*, at least 50 zoöspores have been calculated to be present in the sporangium figured by Nowakowski (l.c. Pl. 6, fig. 10*a*), while 10 is usually the maximum number formed in *C. Nowakowskii*. The turbinate cells of both species are strikingly similar in both size and appearance.

A final point of distinction between the two, which is, however, of doubtful importance, might be added. Our fungus was a virulent parasite of *Spirogyra*, *Oedogonium* and *Coleochaete*.[2] On the other hand, *C. tenue* was found in decaying cells of *Acorus*, *Iris*, *Glyceria*, and in the slime of *Chaetophora*. It might be remarked in this connection that in *C. Nowakowskii* it was possible in hanging drop cultures to observe zoöspores produced by sporangia in *Spirogyra* cells infect *Oedogonium*, form sporangia in the latter host, and produce zoöspores which in turn infected *Coleochaete*. This point is mentioned in view of the fact that some recent investigators have stressed the fact, entirely unsupported by experimental evidence, that species of chytrids are confined to a single host, and, consequently, forms found on different hosts are different species.

Inasmuch as our fungus possessed features which distinguish it from *C. tenue*, it is considered a new species. The name *Nowakowskii* is selected in order that one might be recalled who, in the early days of mycology, conducted classic researches on this group.

[1] *C. polystomum* Zopf (7), while possessing affinities with our organism by reason of its orange pigmentation, is at present so imperfectly known that consideration of it in this connection is not possible.

[2] It has recently (1931) been found in dead *Utricularia* plants in the greenhouse of Dartmouth College, Hanover, N. H.

Cladochytrium Nowakowskii n. sp.[3]

Mycelium intramatrical; filamentous; very tenuous; branching generally dichotomous; possessing septate turbinate cells. Sporangia spherical or pyriform; 13–18 μ in diameter (average 15 μ); with apophyses; each with a discharge tube of varying length; proliferating, the discharge tube in secondary sporangia often penetrating the previous sporangial wall. Zoöspores, 3–15 in a sporangium; delimited within the latter structure; discharged successively through the discharge tube to the outside of the host where they form a motionless, spherical mass surrounded by a vesicle; 7.3 μ in diameter; each with an orange colored[4] oil globule and a single, posterior cilium. Resting spore (?) spherical, thick walled; 14.4 μ in diameter; content colorless, possessing a single orange oil globule; surrounded by a thin, collapsed outer wall; method of formation unknown; germination unknown. Parasitic on *Spirogyra crassa*, *Oedogonium* spp. and *Coleochaete* sp., Cold Spring Harbor, L. I., New York.

(?) Nowakowskiella obscura n. sp.

This fungus was found saprophytic in male strobili of pine which had been used to "bait" a water culture of *Sphagnum* obtained in a bog near the Third Lake, C. S. H., in September, 1927. This water culture was maintained for several years in the Laboratories of Cryptogamic Botany at Harvard, while the writer was a student there, and yielded a number of interesting forms.

As is characteristic of both *Cladochytrium* and *Nowakowskiella*, the mycelium in this organism is quite variable in form and size. The portion which ramifies between the tightly packed pollen grains in the pollen sacks is not only filamentous but is characterized by the possession of frequent spherical, fusiform, and irregular swellings. The mycelium may be considerably expanded at the juncture of the branches and may also have septate turbinate cells similar to those of *Cladochytrium Nowakowskii* (fig. *B*). These cells, however, in contrast to *Cladochytrium*, are only rarely formed. The extramatrical mycelium which bears the sporangia is rather tenuous and possesses large, non-septate, fusiform swellings, similar to those found in other species of the genus (fig. *A*).

Sporangia are borne singly at the apices of the extramatrical hyphae. These are spherical in shape, about 42 μ in diameter, and possess a definite apophysis at the point of attachment of the hypha (fig. *A*). Zoöspores are cleaved out of the colorless content of the sporangium in the manner described for the preceding fungus. In contrast to *Cladochytrium Nowakowskii*, however, many more zoöspores were produced and further, these possessed colorless oil globules.

The discharge of the sporangium is accomplished in a different fashion from that of other species of *Nowakowskiella*. During the formation of the

[3] After this paper went to press, the writer had an opportunity to examine the ms. of Dr. Karling's paper on *C. replicatum* n. sp. (Amer. Jour. Bot. 18: 526–557). It is more than probable that we are both dealing with *C. polystomum* Zopf.

[4] Cadmium orange Ridgway.

spore initials there is produced on the sporangium, either apically or somewhat laterally, a refractive papillum. Upon the discharge of the zoöspores there is formed what might be described as a flattened, flask shaped vesicle at the position of the papillum, apparently derived from the latter structure. The zoöspores, at least a hundred in number, swim into the vesicle and continue to swarm in a vigorous fashion for 10–15 minutes until one of them ruptures the membrane and they swim away (fig. *C*). According to the descriptions of Nowakowski (5), Butler (1), Matthews (3), and others, it is usual for the zoöspores of *Nowakowskiella*, after escaping from the sporangium by the dehiscence of an operculum, to remain in a motionless mass at the mouth of the sporangium. In view of these facts, the fungus under consideration is of considerable interest.

Proliferation of the sporangia occurs in this organism although not to the extent observed in *Cladochytrium Nowakowskii*. In proliferated sporangia, where several superimposed walls were present, a fact was brought out which had been suspected in young, non-proliferated ones; namely, that the sporangium wall is not colorless, as in other species of the genus, but brown. A further distinctiveness is lent to proliferated sporangia by the characteristic collapse and wrinkling of the old sporangial walls (fig. *A*).

The zoöspores are spherical, 4.2 μ in diameter, and possess a central, colorless oil globule. There is a single, posterior cilium about 24 μ long which by its lashing propels the zoöspore in a manner similar to that described for *Cladochytrium Nowakowskii* (fig. *D*). However, in the present species no amoeboid motion was observed, although the zoöspores frequently came to rest on various substrata. Ultimately the zoöspore comes to rest, the cilium is apparently absorbed, and the body increases to a diameter of about 7.2 μ. It then produces a rather thick, hyaline tube which elongates, becomes more tenuous, and branches dichotomously (figs. *E*, *F*). A narrow fusiform swelling is often formed on this limited mycelium. Upon coming into contact with a pollen grain, other more tenuous branches are formed near the point of contact, which presumably penetrate the substratum. The extreme tenuity of these rhizoidal branches and the opaqueness of the walls of the pollen grains make it impossible to follow the fungus within them.

A few resting spores were found, but unfortunately the scantiness and maturity of the material precluded the possibility of observing their method of formation. The spores were borne terminally on stouter portions of the mycelium, were 21–50 μ in diameter, and possessed a dark brown, rather thick, rough wall (fig. *G*). The content, while usually uniformly densely granular, possessed in several instances regularly placed vacuoles, the latter approximately the size of the zoöspores. Although these resting spores were observed for some time, they did not germinate.

From the taxonomic standpoint, this fungus presents several points of interest which, however, will be dealt with only briefly in this paper. It is

realized that the absence of an operculum would seem to preclude the possibility of this fungus being placed in *Nowakowskiella*, as now understood. Yet for reasons which will be taken up in connection with generic concepts in a subsequent publication, the form will be more or less arbitrarily placed in this genus at this time.

From the two species of *Nowakowskiella* previously described, namely *N. elegans* (Now.) Schröter (6) and *N. ramosa* Butler (1), our fungus seems quite distinct. In *N. obscura*, the sporangial wall is brown, a character separating it from both of the aforementioned species. It is further distinguished from these forms by the absence of an operculum and by the behavior of the zoöspores after emergence from the sporangium. No resting spores have been observed in *N. elegans* either by Nowakowski or in recent years by Matthews (3), even though the latter writer apparently possessed an abundance of material and surely would have observed them if they had been formed under ordinary conditions. In *N. ramosa*, resting spores were formed which possessed a smooth outer wall, in contrast to the roughened ones of our fungus. Further, the spores of Butler's species were somewhat smaller (18–25 μ in diameter cf. 21–50.4 μ), and the formation of a type of "parenchymatous" tissue described for *N. ramosa* was not observed in *N. obscura*.

Since the fungus under discussion appears to be distinct from the other known congeneric forms, it is proposed to name it *N. obscura*, the specific name referring to the dark color of the sporangium wall and the resting spore.

(?) **Nowakowskiella obscura** n. sp.

Mycelium exceedingly irregular in size and shape; ramifying between pollen grains; possessing numerous broadly fusiform swellings as well as septate turbinate cells. Sporangia brown walled; spherical or sub-spherical; 42 μ in diameter; with apophyses; proliferating, the walls of the proliferated sporangia generally wrinkling; each possessing an apical or sub-apical papillum which by its gelatinization allows the zoöspores to escape into a vesicle where they swarm. Zoöspores spherical; 4.2 μ in diameter; possessing a central, colorless oil globule and a single, posterior cilium. Resting spores 21–50.4 μ in diameter; each possessing a thick, rough, brown, outer wall. Germination not observed. Saprophytic on male strobili of *Pinus*, in water culture from Cold Spring Harbor, L. I., New York.

Summary

Two new species of aquatic Phycomycetes, *Cladochytrium Nowakowskii*, a parasite of green algae, and *Nowakowskiella obscura*, a saprophyte of staminate pine cones, have been found in the vicinity of Cold Spring Harbor, Long Island.

The morphology and life history of these fungi are described. Certain significant differences in the non-sexual reproduction of *Nowakowskiella obscura* from congeneric species are described. The ability of *Cladochytrium*

Nowakowskii to parasitize several genera of green algae as well as to grow on corn-meal agar is noted. A brief taxonomic consideration of the two fungi is also included.

The writer wishes to thank Prof. W. H. Weston, Jr., of Harvard University for his kindness in critically reading over the manuscript and for making certain suggestions.

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EXPLANATION OF PLATE XLV

All figures were drawn with the aid of the camera-lucida. The approximate magnifications are given in each instance.

? *Nowakowskiella obscura* n.sp.

FIG. *A*. Habit of plant with a discharged, proliferated sporangium and an immature one. Former in optical section. × 580.

FIG. *B*. Mycelium ramifying between pollen grains. The irregularity of the hyphae and two septate turbinate cells are shown. × 50.

FIG. *C*. A discharging sporangium, in optical section. The zoöspores are swarming at a rapid rate within a broadly conical vesicle formed, in this instance, at the apex of the proliferated sporangium. A smaller apophysis is present than in the proliferated sporangium in figure *A*, variation in this respect being characteristic of the fungus. × 500.

FIG. *D*. Zoöspore. × 500.

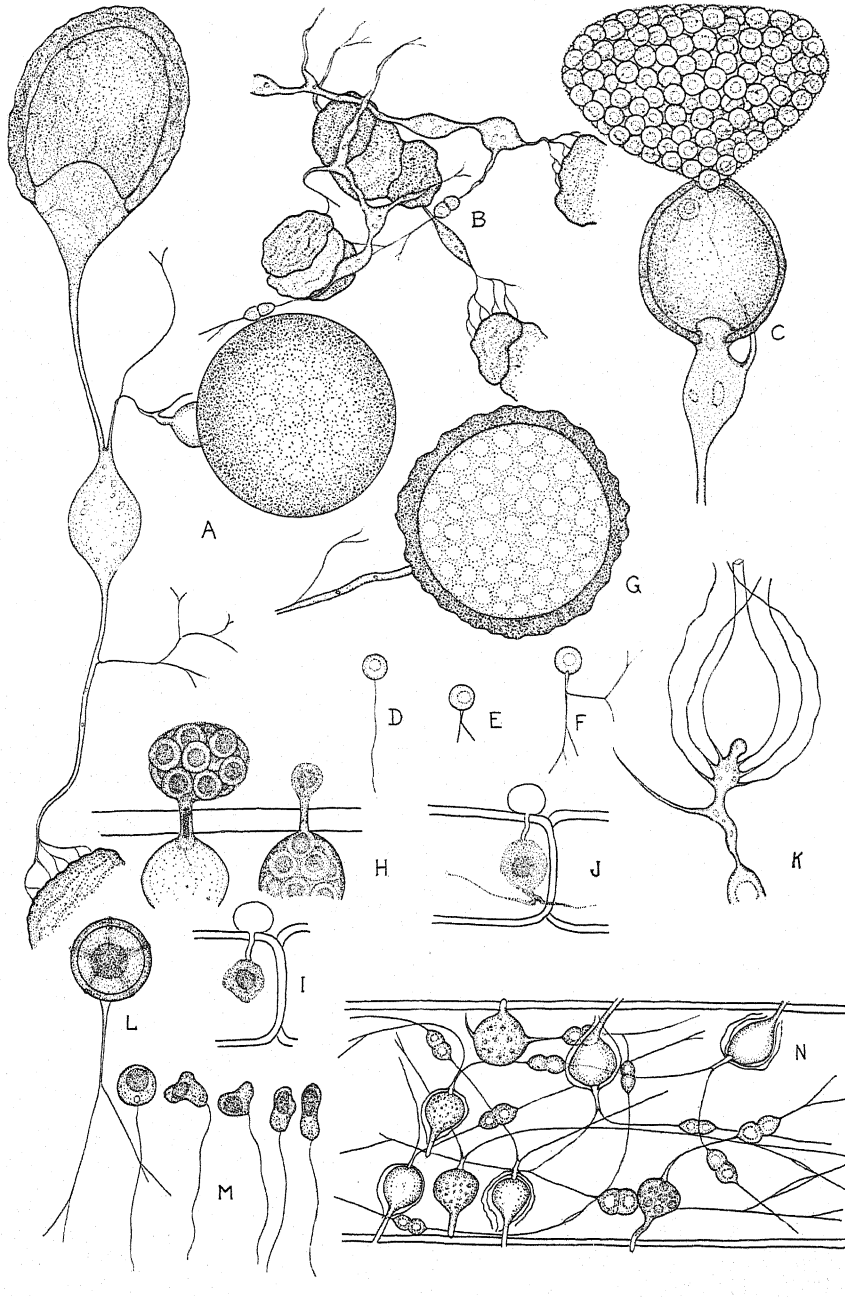
FIGS. *E*, *F*. Stages in the germination of the zoöspore. × 500.

FIG. *G*. Resting spore, in optical section, showing the rough, brown wall and vacuolate content. × 580.

Cladochytrium Nowakowskii n.sp.

FIG. *H*. Two discharging zoösporangia. The zoöspores are ejected to the outside of the alga, where they form a motionless group, surrounded by an exceedingly tenuous vesicle. No cilia can be observed, probably because of their tenuity. × 580.

FIG. *I*. Infection of an *Oedogonium* filament by a zoöspore. The cystospore has produced a narrow germ tube which has penetrated the host wall and through which the protoplasm of the spore has passed into the interior. × 580.



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FIG. *J*. Later stage of infection. A tenuous mycelium is being established within the *Oedogonium* filament. × 580.

FIG. *K*. A discharged sporangium showing the remains of three successive sporangial walls, each with a different point of egress of the discharge tube. × 1125.

FIG. *L*. Structure found in a single instance in an infected *Spirogyra* cell. An outer, very tenuous, wall has collapsed in a characteristic fashion around an inner thick walled spore which possesses in its protoplasm an orange globule. The method of formation of this body and its relationship to the *Cladochytrium* are not known. × 580.

FIG. *M*. Zoöspores, showing at extreme left the shape during swimming, and in the other figures the shapes taken during amoeboid movement. × 580.

FIG. *N*. Habit of the fungus in a cell of *Spirogyra*. Sporangia in various stages of maturation are shown, as well as the extensive mycelium and its biseptate turbinate cells. × 260.

LABORATORY CULTIVATION OF MYXOMYCETE PLASMODIA

FRANK L. HOWARD

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The need for Slime Mould plasmodia in large quantities for research and instructional purposes is frequently felt in biological laboratories. Several substrata for their cultivation have been reported in the literature, but after trial the writer finds that they are inadequate for providing sufficient quantities of plasmodium for laboratory experimentation when needed. The first nutrient substratum was introduced by Lister (6) in 1888 as a result of his observation that the plasmodium of *Badhamia utricularis* would digest and assimilate the fruit-bodies of certain Hymenomycetes. Since then, additional fungous sporophores have been used as a source of food for plasmodia by various investigators and recently the writer (4) has used mycelium with success. Of the fungous material tried by the writer certain white-spored agarics have provided the best substratum for the most rapid growth of plasmodia of such mycophagous species as *Physarum polycephalum*, *P. viride*, etc.; for example, a large pileus of *Amanita muscaria* will be completely digested by such species as these within twenty-four hours. Certain distinct disadvantages, however, are encountered in the use of fleshy fungous sporophores in that they must be renewed almost daily and they are only available at infrequent intervals during the year.

In order to carry on cytological and developmental studies, it was soon apparent that a method of culturing plasmodia just as one does fungi in fairly large quantities on a common laboratory medium needed to be developed. After considerable experimentation the writer has devised a method of growing plasmodia on oat agar[1] in petri plates which seems to meet this need and to overcome the disadvantages encountered in the use of fungous sporophores.

[1] An unpublished manuscript on the cultivation of myxomycetes, comprising work done during 1918–20 by Miss Mary E. Currie (now Mrs. Gordon Edwards) at the University of Toronto, under the direction of Dr. J. H. Faull, has just been made available to the writer. Miss Currie cultivated plasmodia from spores sown upon various media and concluded that oat decoction agar and corn seed decoction agar were best suited to the development and maintenance of plasmodia. She obtained plasmodia from 18 of 25 species whose spores germinated upon these two agars; from 16 of 21 species germinated upon corn seed agar, and from 4 of 15 species germinated upon rolled oat agar. Miss Currie recommends using 25 grams of ground sweet corn (Golden Bantam) and 25 grams of ground field corn (Red Cob Ensilage) to one liter of water, and, after autoclaving one hour at 15 pounds pressure, adding 25 grams of agar to the filtered decoction which is then sterilized and poured into sterile petri plates. Although Miss Currie preferred corn seed decoction agar as a medium, she points out that the plasmodia of some species make a more rapid growth upon rolled oat decoction agar.

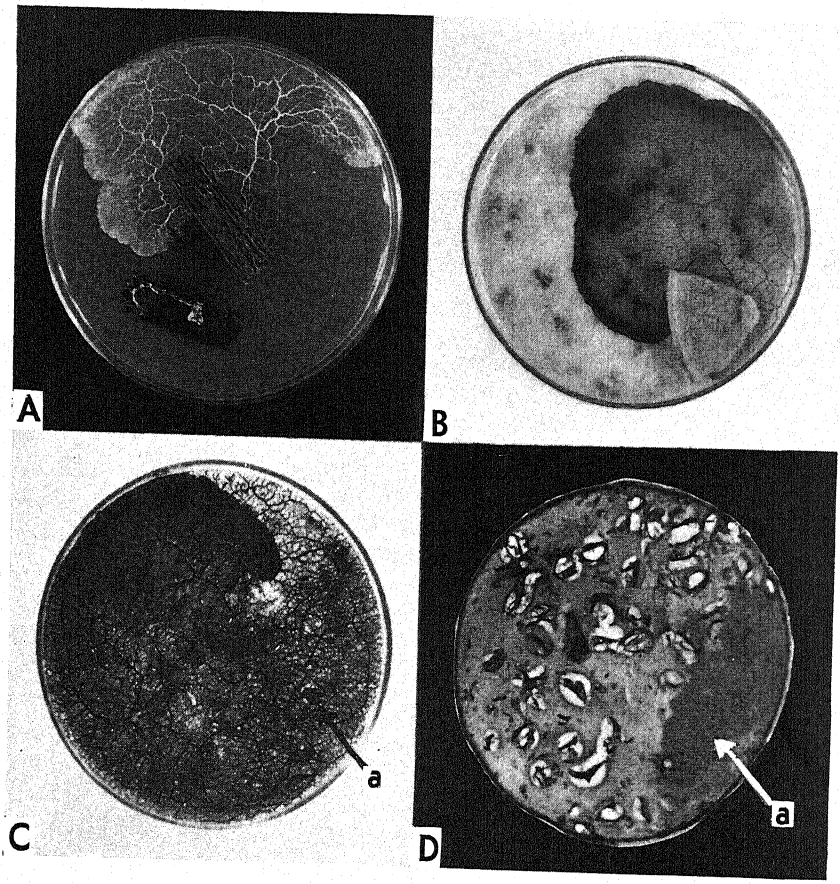
Before the use of oat agar was developed, a wide range of nutrients was tried. Since Knop's agar plus dextrin or sucrose has been reported (2, 3, 8) as furnishing the necessary nutrients for the complete life cycle these media were tried, but on them the plasmodia grew slowly if at all. Observations were then made of the growth of plasmodia upon natural and compounded media. Little or no growth was evident on: potato plugs, gelatin, fresh cooked beef, egg white, hardwood shavings, tea decoction agar, wood decoction agar, soil decoction agar, horse dung decoction agar, and proteose-peptone agar. Fair growth of the plasmodia was obtained upon moist mashes of sprouted and autoclaved oats, corn, and lima beans. Yellow corn meal agar also supported a fair growth of the plasmodia. Best development of the plasmodia, however, was obtained on rolled oat agar, carrot decoction agar, and autoclaved egg yolk.

In general, the features characterizing a medium for the satisfactory cultivation of plasmodia are: (1) that the plasmodia should grow vigorously on it; (2) that it should provide sufficient support for the plasmodia to be easily transferred; (3) that renewal of the medium weekly or fortnightly should be sufficient; and (4) that the ingredients should be inexpensive and available throughout the year. Egg yolk is, therefore, impractical as a medium because of its high cost, its furnishing of insufficient support for the plasmodia when transferring, and its rapid decomposition which necessitates almost daily transfer of the plasmodia. Carrot decoction gelatin has previously been used with success by Skupienski (8) but the writer's experience with this agar is that the plasmodia do not make nearly as rapid a growth upon it as upon oat agar. The writer has tested oat agar as a nutrient substratum for some thirty different collections of yellow and white colored plasmodia and has found that it fulfills all of the requirements of a satisfactory medium as listed previously (text fig. 1, *B*, *C*).

The oat agar is prepared by cooking 30 grams of rolled oats, 15 grams of agar, and one liter of water ten to fifteen minutes in a double boiler. The gruel is poured into flasks and then autoclaved at 15 pounds pressure for fifteen minutes. Unless the cooking preceding autoclaving is done, the cotton plugs of the flasks are usually blown out during autoclaving. The time of cooking the gruel should be minimized since in most cases the plasmodia seem to prefer to absorb the nutrients directly from the oat kernel (text fig. 1, *C*) instead of from suspension in the agar. It is well to avoid excessive water of condensation in the petri plates by cooling the oat agar to almost the point of solidification before pouring it into the sterile plates.

Upon plates thus prepared plasmodia will remain in an active condition when placed at temperatures from 7° C. to 32° C. although they seem to grow most rapidly between 20° and 26° C. It is also observed that plasmodia seem to prefer a slightly acid medium. Plasmodia of *P. polycephalum* will even tolerate an acidity of oat agar as great as pH 4.8 although some other species seem to be less tolerant.

The plasmodia to be grown in culture may be collected in nature either as active plasmodia or as desiccated plasmodia (sclerotia). Sclerotia are rather inconspicuous and for this reason it is chiefly active plasmodia which



TEXT FIG. 1. *A*, yellow plasmodium moving from wood to non-nutrient agar in a petri plate after transportation to the laboratory from the field. *B*, plasmodium moving from transfer piece to fresh oat agar. *C*, plasmodium after 72 hours growth on oat agar; note where the oat kernel has been eaten from the agar (*a*). *D*, same plasmodium desiccated on the oat agar to form a sclerotium (*a*). All × ½. Figures *B* and *C* were photographed on orthochromatic plates and *A* and *D* on panchromatic plates.

are gathered. Experience has shown that even during dry seasons plasmodia may be found under the bark of fallen tree trunks and branches. Although decorticated logs and the surrounding forest debris may be as dry as tinder, logs which are bark-covered retain sufficient moisture to allow the plasmodia to remain in an active state within them.

Plasmodia, when once discovered, present the difficult problem of transportation to the laboratory uninjured because of their delicate structure

which renders them likely to be damaged or even killed. A method of carrying the plasmodia in petri plates has been devised and successfully used to overcome this difficulty (text fig. 1, *A*). A thin layer of 1.5 percent agar is poured into sterile petri plates and each plate is then wrapped in paper to avoid breakage. In the field the piece of wood, leaf, or other substratum supporting the plasmodia is transferred to the petri plate which permits the plasmodia to be carried to the laboratory safely because the agar furnishes a sufficiently high humidity and the glass prevents crushing. Within a few hours the plasmodia will leave their natural substrata and move onto the agar (text fig. 1, *A*). After several frequent successive transfers on non-nutrient agar they will be reasonably free from contaminating organisms and may then be transferred to oat agar, upon which, the plasmodia thus obtained will grow copiously (text fig. 1, *C*), and may eventually form normal sporangia. In this way the writer has been able to make certain an unfailing supply of active normal plasmodia.

In addition to the need of growing abundant material for experimental purposes, a second need which has been experienced is the insuring of a supply of plasmodia for future use. Although plasmodia can be kept by repeated transfer in an active condition from year to year, sclerotia meet this need more advantageously. Several investigators have in the past laid aside and distributed sclerotia which they collected in the field or grew on natural substrata. The writer has found that plasmodia grown on oat agar can be desiccated (text fig. 1, *D*) and kept in an inactive state for future needs. Sclerotia are reported to have been reactivated after storing three years by Lister (6), after seven years by Schinz (7), and after twenty years by Léveillé (5). It is the writer's experience that the percentage of those capable of being revived decreases rapidly after one or two years. This length of time, however, is sufficient to carry over plasmodia from one college year to the next or from one piece of research to another. Thus we now have a method of growing large quantities of plasmodia which may be inactivated and kept for future needs.

The desiccation is accomplished by merely exposing the plasmodia on the substratum to the air in order that there may be a slow loss of water. Brandza (1) has made extensive observations upon myxomycete sclerotia and finds that "sclerotization" of the active plasmodia is brought about by the slow loss of water especially in a calm atmosphere to below the minimum for normal development. Too rapid water loss causes the plasmodia to become of a horn-like consistency, a condition from which they are incapable of being reactivated. In trials made by the writer normal sclerotia were revived to active plasmodia within four to twenty-four hours by placing the sclerotia under moist conditions but taking care not to cover them with water, for most plasmodia are strongly aërobic and soon die if submerged.

Although plasmodia of Myxomycetes have been used to a limited extent for the investigation of living unwalled protoplasm and its chemotropic,

hydrotropic, and rheotropic responses, or of reversible protoplasmic streaming and its relation to movement, such important and interesting study has always been greatly handicapped hitherto by the lack of a dependable supply of adequate normal material.

Summary

The need of active myxomycete plasmodia in large quantities for research and instructional purposes has been met by the cultivation of plasmodia on rolled oat agar. Moreover, a future supply of plasmodia is assured by desiccating the plasmodia thus cultivated on the oat agar to sclerotia which may be reactivated as needed by the addition of moisture. The methods of collection, culture, and maintenance, which have proved so successful in providing abundant material for the writer's own work, are presented in order that others may have available similar vigorous plasmodial material for the investigation of many significant problems.

This paper was written while the author was the holder of a National Research Fellowship in the Biological Sciences. He wishes to thank Professor Wm. H. Weston, Jr. for the keen interest he has shown in the progress of this work and for the helpful suggestions he has made in connection with the preparation of this paper.

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CYTOLOGICAL EVIDENCE OF GENETICAL RELATIONSHIPS IN *OENOTHERA*

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Recent cytological and genetical studies on *Oenothera* have revealed the existence of close correlations between chromosome behavior in diakinesis, on the one hand, and genetical relationships between the complexes on the other. It is the purpose of this paper to summarize briefly the data which point to the presence of such correlations, to suggest a possible explanation for their existence, and to indicate further lines of study which they seem to render possible.

The correlations with which I shall deal are three in number:

1. Plants with identical genetical make-up have in general identical chromosome configurations in diakinesis.

2. The second correlation has to do with certain gene complexes which reside in different races, but are nevertheless essentially identical genetically, or closely related. When essentially identical, such complexes give identical chromosome configurations in combination with a given third complex; when closely related, they give similar configurations when so combined.

3. Complexes which are closely related genetically yield, when combined with *each other*, configurations in which most or all of the chromosomes are paired. Those which are more distantly related give configurations in which more of the chromosomes are involved in circles.

These correlations will be considered in turn.

1. *Plants with identical genetical make-up have in general identical chromosome configurations in diakinesis.*

The data which support this conclusion are drawn from three sources: (*A*) from the results of repeated cytological examination of pure pedigreed lines, involving numbers of individuals, grown in different years and studied by different persons; (*B*) from examination of reciprocal hybrids; (*C*) from examination of "synthetic species."

A. Of the spontaneous forms which have been studied genetically, the following have been adequately examined more than once from the cytological standpoint: *Lamarckiana*, *biennis*, *muricata*, *suaveolens*, *Hookeri*, and *Chicagoensis*. Several strains of *Lamarckiana* emanating from both Holland and Sweden have been examined and published upon in several years by Håkansson (1926, 1928), Illick (1929), Weier (1930), and myself (1925, 1929). All material has been shown to have a circle of 12 and 1 pair. (Boedijn's material seems to have been inadequate, so that he missed the circle of chromosomes.) *Biennis* has been studied by 4 workers, Emerson (1924),

Valkanover (1926), Kihara (1927), and myself (1923, 1926, also Cleland and Oehlkers 1930). Although derived from several sources, and collected in different years, this species has shown in every case a circle of 6, circle of 8. *Muricata* has been examined by the writer at 4 different times. The material collected in 1919 and 1920 formed the basis of an earlier paper (1926). In addition, a culture grown in Philadelphia in 1926 from seed sent direct from de Vries, as well as cultures of *r-muricata* and *R-muricata* collected at Tübingen in 1927, have been examined. In every case, a circle of 14 has been found. *Suaveolens* has been found by both Illick (1929) and myself (1928, also Cleland and Oehlkers 1930) to have a circle of 12, and 1 pair. (I have re-examined this species in 1929, with similar results.) *Hookeri* de Vries has been shown to have 7 pairs by Schwemmle (1924), Weier (1930), and myself (1928). Material grown by the present writer in 1929 showed also 7 pairs. *Chicagoensis* has been collected by the writer in 3 different years, in 3 different countries, with a circle of 12 and 1 pair present each time. (A detailed account of this species and certain of its hybrids is in course of preparation.)

Altogether, there have been examined a goodly number of plants of all these species, belonging to cultures grown in different years, and in different localities; and in most cases, the studies have been made by more than one person. The constancy of chromosome behavior has been as striking as the constancy in breeding behavior. It may be taken as a general rule that plants in pure line, with the same genotypic composition, have identical chromosome arrangements.

B. Further evidence of the correlation under consideration may be drawn from a study of reciprocal hybrids, using the term to mean hybrids which are not only reciprocally obtained, but are also identical from the standpoint of complex-combination. (The term has been more loosely used in some cases in connection with *Oenothera* studies,—*e.g.*, Gates and Sheffield, 1929.) Eighteen cases of true reciprocal hybrids have been investigated by the writer, some of them as yet unpublished, and in every case the reciprocals have been found to have identical chromosome configurations.

C. Complex-combinations identical or practically identical in genetical composition with spontaneous forms may sometimes be formed synthetically by appropriate crossing. Such synthetic combinations have so far shown the same chromosome configurations as the wild forms with which they are identical. Thus, one of the complex-combinations which results from the cross *biennis* × *suaveolens* is *albicans·flavens*. *Albicans* being common to both *biennis* and *suaveolens*, the hybrid is in reality practically or wholly identical with the species *suaveolens* and indistinguishable from it phenotypically, although a so-called interspecific hybrid, and having its *albicans* derived from *biennis* instead of from *suaveolens*. It has the circle of 12 and 1 pair characteristic of *suaveolens*, and when selfed, it breeds true for this configuration (new cytological data).

Another illustration is to be found in the cross *biennis* × *Hookeri*. This results in twin hybrids, *albicans*·^{h}*Hookeri* and *rubens*·^{h}*Hookeri*. When selfed, both of these hybrids produce two types of progeny in F_2, of which one type in each case has the composition ^{h}*Hookeri*·^{h}*Hookeri*, and is therefore from the genetical standpoint practically identical with *Hookeri*. Like *Hookeri*, such F_2 plants have 7 pairs of chromosomes (new data).

Still a third instance involves the re-synthesis of *biennis* (*albicans*·*rubens*). When *Chicagoensis* ♀ is crossed with *biennis* ♂, the normal hybrid produced is *excellens*·*rubens*. However, the metacline hybrid, *punctulans*·*rubens*, appears in a small percentage of cases (configuration is circle of 6, circle of 8). When this is crossed back to *biennis* (*biennis* × metacline), the following complex-combinations are theoretically possible, and are actually realized: *albicans*·*punctulans*, *albicans*·*rubens*, and *rubens*·*punctulans*. The second of these is a resynthesized *biennis*. It has the circle of 6, circle of 8 characteristics of this species (unpublished data).

These are the only cases where resynthesized species have been examined cytologically, so far as I know, and in each instance the reconstituted species has the configuration characteristic of the spontaneous species.

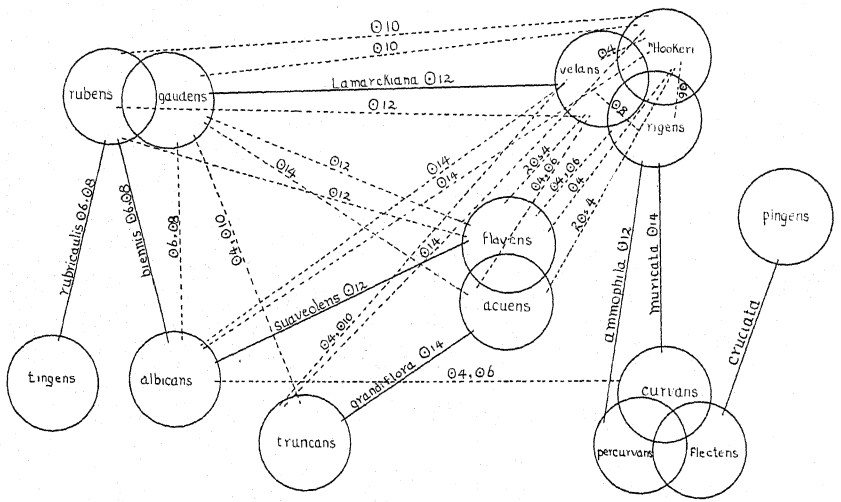
Taking it all in all, the sum total of available data from a cytological study of pure lines, reciprocal hybrids, and "synthetic species" indicates that plants identical in genetical composition have identical chromosome configurations.

2. The second correlation may be stated as follows: Complexes are known which reside in different races, but are nevertheless essentially identical genetically, or closely related. *When essentially identical, such complexes give identical chromosome configurations in combination with a given third complex. When closely related, they give similar configurations when so combined.*[1]

For evidence in support of this statement, we may turn to text figure 1. This is based upon a diagram by Hoeppener and Renner (1928), in which these authors have portrayed, in a necessarily rough and tentative way, the relation that appears to exist between various complexes, as determined by genetical analyses, mainly by Renner. Complexes are represented in the diagram by circles. Those close together or overlapping are closely related genetically, *i.e.*, they hold most of their known genes in common. Those lying at a distance are more distantly related, *i.e.*, they are heterozygous in respect to more or most of their known genes. Those connected by solid lines are normally associated to form some one of the spontaneous forms. The original diagram of Hoeppener and Renner included only the circles and solid connecting lines, which were in the same positions shown in our

[1] It is to be understood that this correlation applies only in cases where the complexes in question are passed on to the next generation essentially intact by the plants in which they are found. However, complexes are passed on essentially intact in all species so far studied genetically. It is only in the case of certain hybrids where they become more or less broken up.

adaptation of it. Upon this diagram I have superimposed the chromosome configurations given by the various complex-combinations, as far as they are certainly known,[2] indicating configurations of artificially produced complex-combinations by dashed lines.



TEXT FIG. 1. The diagram of Hoeppener and Renner (1928) showing tentatively the relationships between certain complexes of *Oenothera*, upon which have been superimposed the chromosome configurations produced by the various complex-combinations, so far as known. Complexes connected by solid lines are normally associated to form spontaneous races. Those connected by dashed lines together represent artificially produced hybrids whose chromosome configurations have been determined. For discussion, see text.

The following facts will serve to bring out the correlation:

Rubens and *gaudens* are almost identical genetically, according to Renner (Hoeppener and Renner, 1928, p. 22), though *rubens* is found in *biennis*, and *gaudens* in *Lamarckiana*. In view of their great similarity, it is interesting to find that in every case where both are known cytologically in combination with a given third complex, they give the same configuration. Thus, both give the same configuration with *velans* (circle of 12, 1 pair), with *[h]Hookeri* (circle of 10, 2 pairs), with *flavens* (circle of 12, 1 pair), with *albicans* (circle of 6, circle of 8); as well as with *excellens* (circle of 10, 2 pairs) and *punctulans* (circle of 6, circle of 8), which do not appear in the diagram. As yet no cases are known where *rubens* and *gaudens* fail to give identical configurations with a third complex.

[2] I have not included configurations reported by Gerhard (1929) for the reason that he reports a different configuration for *grandiflora*, as well as for certain of its hybrids from those that I have found. When Gerhard's paper appeared, I went over my material again to make certain that I had made no mistake, and found that my results were correct. I have since reëxamined *grandiflora* (grown 1930), and find this material to have a circle of 14, as I had previously reported (Gerhard reported a circle of 12). The possibility that Gerhard has studied material which is cytologically divergent from that represented in the diagram makes it advisable to omit his reported configurations from the diagram, pending confirmation.

Albicans is another complex of the same sort. It is found in two species, being practically identical in *biennis* and *suaveolens*. It is known to give identical configurations with *flavens*, *punctulans*, and *velans*, irrespective of which species it is derived from.

These are the only cases in which complexes identical or nearly identical in different species have been compared from the standpoint of cytological behavior, when derived from the different species. Turning now to closely related but not identical complexes, we may consider first of all *velans* and *hHookeri*. With *rubens* and *gaudens*, *velans* gives a circle of 12, *hHookeri* a circle of 10. With *albicans*, both give a circle of 14. With *flavens*, *velans* gives 2 circles of 4, *hHookeri* one circle of 4. With *acuens*, *velans* gives a circle of 4, circle of 6, *hHookeri* 2 circles of 4. With *rigens*, *velans* gives a circle of 8, *hHookeri* a circle of 6. In every case, these 2 complexes yield configurations with another complex which could not be more alike, without being actually identical, except in the one case where they are actually identical.

Another pair of complexes said to be closely related are *acuens* and *flavens* (Gerhard, 1929, p. 288). With *gaudens*, *flavens* gives a circle of 12, *acuens* a circle of 14. With *hHookeri*, *flavens* gives a circle of 4, *acuens* 2 circles of 4. With *velans*, *flavens* gives 2 circles of 4, *acuens* a circle of 4, circle of 6. Again the configurations given by the two complexes could not be more alike without being identical.

Still another pair that are rather closely related are *albicans* and *tingens* (Rudloff 1929, p. 226). The latter gives the same configuration with *rubens* as the former.

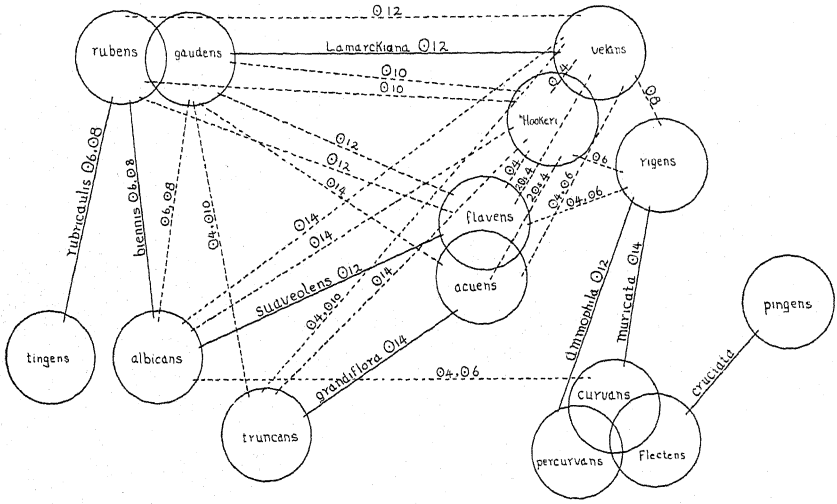
Curvans and *percurvans* are very similar also (Hoeppener and Renner 1928, p. 23). One gives a circle of 14, the other a circle of 12 with *rigens*.

So it will be seen that in the case of complexes which have been to some extent analyzed genetically, those which are essentially identical, but reside in different races, give identical configurations with a third complex; and those which, while not identical, are nevertheless closely related, give very similar configurations.

3. The third correlation is as follows: *Complexes which are closely related genetically yield, when combined with each other, configurations with most or all of the chromosomes paired. Those which are more distantly related give configurations in which more of the chromosomes are involved in circles.*

For evidence of this we may again turn to text figure 1. It will be seen that on the whole complexes which are far apart in the diagram show larger circles when combined than those which are closer together. When it is considered that Hoeppener and Renner's diagram only purports to be tentative and based upon incomplete evidence, it is remarkable how close is the correlation. It would be even more striking if the position of certain complexes in their diagram were slightly altered, which, in view of the tentative nature of the diagram, seems admissable. If *hHookeri* were placed below

velans, and if *rigens* were separated slightly from *h Hookeri* and *velans*, being slightly nearer to *h Hookeri* than to *velans* (see text figure 2), the relative distances would correspond even better to the chromosome configurations; and I am inclined to think that the modified diagram would as well express the genetical relationships as the original one.



TEXT FIG. 2. A slight modification of text figure 1. *h Hookeri* has been placed below *velans*, and *rigens* has been displaced slightly to the right. The correlation between circle size and genetic similarity between complexes is somewhat better brought out by the use of text figure 2, which from the genetic standpoint is probably as accurate as text figure 1.

On the whole, it would seem that the correlation between genetical relationship and chromosome configuration is a noticeably clear one. So far as the data go, the more closely complexes are related to one another, the smaller are the circles which they will form when combined, and vice versa.

We come now to a consideration of the reasons for the existence of such correlations between chromosome configuration and genetical relationships between complexes as have been briefly outlined. Various explanations have been offered. I was formerly inclined toward the theory that circle formation was the *result* of the presence of a high degree of heterozygosity on the part of the chromosomes making up the circle, circle size depending upon the number of chromosomes carrying a high proportion of heterozygous genes. A close correlation undoubtedly exists, as I have pointed out above, between circle size and the degree of heterozygosity existing in a given complex-combination. I am inclined at present, however, to consider circle formation not as a result, but as a contributing cause of the presence of a high degree of heterozygosity, and to consider segmental interchange, in turn, as the cause of circle formation. The segmental interchange theory of Belling's has been applied to the *Oenothera* situation by Darlington (1929) and Håkansson (1930); as well as by Blakeslee and the present writer (1930),

who have shown that successful predictions may be made, on the basis of this theory, as regards the chromosome configurations in certain combinations of complexes. The theory of segmental interchange assumes an attraction between homologous parts or ends of chromosomes as the force which causes chromosomes to associate in diakinesis, and postulates a mutual exchange of segments between non-homologous chromosomes as the cause of circle formation.

On the basis of this theory, the above correlations become understandable. Complexes which are identical genetically have as a rule chromosomes with identical end arrangements, and the same complex generally has the same arrangement of ends; plants which are identical genetically, will have the same chromosome configurations because they are combinations of the same complexes. Complexes which reside in different species, but are none the less identical, and give identical chromosome configurations, do so because they have the same arrangement of ends. It is confidently to be expected, therefore, that such complexes (*e.g.*, *rubens* and *gaudens*) will continue to yield identical chromosome configurations with other complexes, so long as their chromosomes continue to possess an identical arrangement of ends. That complexes which are closely related to one another yield similar configurations with other complexes and small circles with each other, is due to the fact that they differ from one another by only one, or at most a very few segmental interchanges, so that their chromosomes have similar though not identical arrangements of ends. In order to understand why closely related complexes should have chromosomes with similar arrangements of ends, it is only necessary to assume that segmental interchanges have been of rare occurrence, at least such as have taken place without the production of deficiencies or duplications resulting in the loss of viability of the resultant zygotes. Granted this assumption, there will have been ample time between any two successive interchanges for gene mutations to have occurred, and in forms with balanced lethals in the circles, for these mutations to have been accumulated, with consequent modification and divergence in the genetic nature of the associated complexes. The fewer the interchanges, therefore, the less the divergence, on the whole.

Thus, two complexes which when combined yield a circle of 4, and 5 pairs, differ from each other, as far as end arrangement is concerned, by but a single interchange. In respect to 5 of their chromosomes they have an arrangement of ends that is identical. It is probable that in most cases such complexes, differing by but a single interchange, have been derived in relatively recent times from a common source, for example:

1·2	3·4	5·6	7·8	9·10	11·12	13·14
1·4	3·2	5·6	7·8	9·10	11·12	13·14

On the other hand, two complexes which yield, when combined, a circle of 14, differ by a number of segmental interchanges (at least 6), for example:

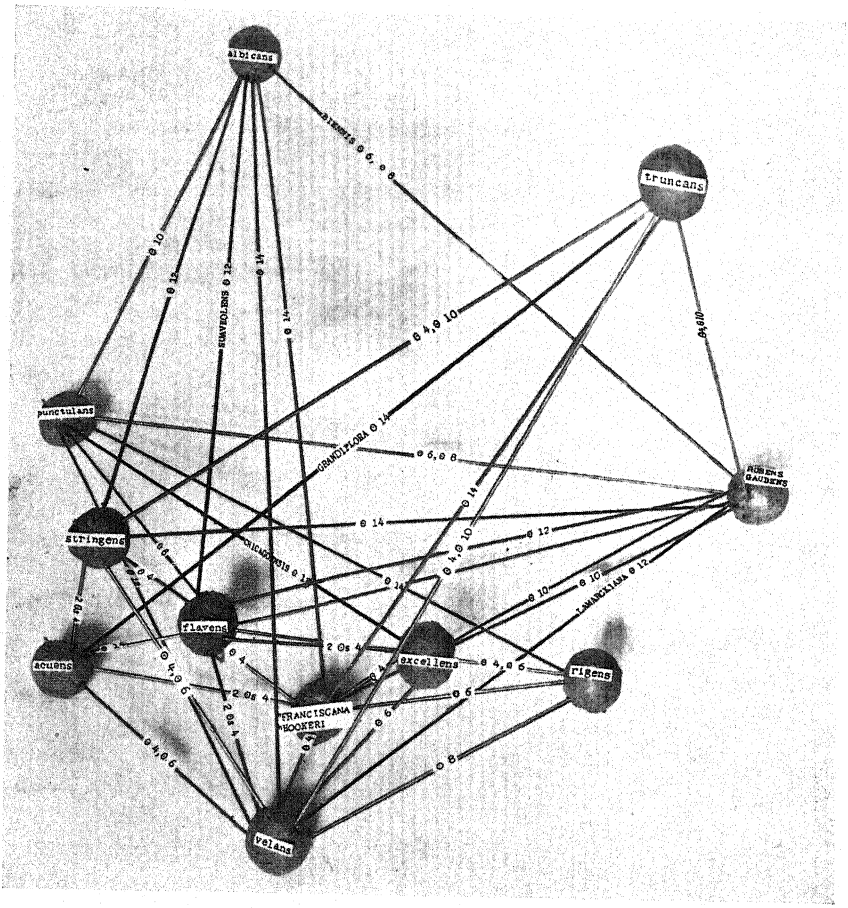
1·2	3·4	5·6	7·8	9·10	11·12	13·14
1·4	3·6	5·8	7·10	9·12	11·14	13·2

The probability is that they have long ago diverged from their common source, and have experienced long histories of genetic modification since that time, accompanied at rare intervals by segmental interchange. Large circles, since they imply a succession of segmental interchanges in at least one of the complexes involved, are evidence of a long evolutionary history since the time when the complexes which together yield this configuration diverged genetically. Small circles conversely indicate a relatively short evolutionary period. In a rough way, therefore, circle size becomes a measure of genetic similarity. Complexes which give with each other large circles are relatively dissimilar genetically (have more heterozygous genes), those which give small circles are relatively similar (have fewer heterozygous genes). One can therefore tell something about the relation between complexes, and hence between species of *Oenothera*, by studying chromosome behavior.

If then it be true that chromosome behavior affords some clue to genetic relationships in *Oenothera*, it ought to be possible to construct a diagram such as Hoeppener and Renner have done, but based entirely upon circle size, a diagram which will express as accurately the *degree* of genetic similarity between complexes as it has hitherto been possible to do through genetic analyses. I have consequently made an attempt at a graphic portrayal of the relation between complexes, ignoring entirely genetic data, and relying solely upon facts with regard to chromosome configurations. Realizing, however, the inadequacy of a diagram drawn in one plane, I have tried instead to construct a model in 3 dimensions which will give perhaps a better idea of the inter-relationships of some of the complexes than a diagram in one plane can do. Such a model is shown in text figure 3.

In this model, the spheres represent complexes. Each individual complex-combination (*i.e.*, species or hybrid) is represented by 2 spheres connected together by a rod. The length of the rod has been determined on the basis of the minimum number of segmental interchanges which would produce from a common source two complexes giving, when combined, the chromosome configuration actually found in the particular complex-combination. Thus, *[h]Hookeri* and *velans*, when combined, give a circle of 4 and 5 pairs. These two complexes differ therefore by but a single interchange. They have consequently been placed one unit of distance apart in the model, and the rod has been labelled "⊙4." Again, *acuens* and *velans* give, combined together, a circle of 4, circle of 6, and 2 pairs. The minimum number of segmental interchanges that would have to occur in a form in which paternal and maternal chromosome complexes had the same end arrangement, in order to produce the end arrangements necessary to give this configuration, is three; these complexes have been placed therefore 3 units of distance apart, and the rod connecting them has been labelled "⊙4,⊙6." Under only one circumstance has this rule regulating the length of rod been departed from. I have felt free in cases where complexes give a circle of 14

with each other, to make the rods connecting these two complexes longer than the minimum of 6 units of distance where necessary, and for the following reason. The larger the circle, the less the liklihood that the two complexes giving this circle are separated by the minimum number of inter-



TEXT FIG. 3. Photograph of a model based entirely upon cytological data, showing relationships between certain complexes. The model includes *h franciscana*, which gives 7 pairs with *h Hookeri* (new data), and also includes *excellens*, *punctulans*, and *stringens*, none of which are found in the diagrams. On the other hand, *tingens*, *pingens*, *curvans*, *percurvans*, and *flectens* are omitted from the model for lack of cytological data. For further explanation, see text.

changes that would give the configuration in question. Smaller circles may arise from larger circles through segmental interchange, just as larger ones can arise from smaller ones. Consequently, the probability is that two complexes giving a circle of 14 with each other are actually separated by more than the minimum number of 6 interchanges. Except for this one deviation,

the rule has been followed implicitly that the length of rod in each case represents the minimum number of interchanges which would produce, from a common source, two sets of end arrangements which, when combined, would give the chromosome arrangement actually present in the particular complex-combination.

No claim is made that the distances separating the various complexes in the model are accurate in other than a relative sense. However, if our assumption is correct that interchanges producing viable forms occur but rarely, and the size of circles is consequently a rough measure of the genetic relationship between complexes, as suggested above, then we are safe in saying that those complexes which are closer together in the model are more closely related than complexes which are separated by a greater distance. Now, when we compare this model with Hoeppener and Renner's diagram, we are impressed by the similarity between the two, as indeed is to be expected, in view of the close correlation which we have seen to exist between circle size and genetic relationship between the complexes. Complexes which are close together in the diagram are close together in the model, and vice versa. The conclusions with reference to degrees of genetic similarity between complexes which have been reached upon the basis of purely genetic analyses are essentially the same as those which one would be led to adopt upon purely cytological grounds.

The extent to which the correlation between circle size and genetic relationship between complexes has been found to hold thus far warrants us in adopting as a working hypothesis the assumption that such a correlation is of widespread application within the genus, and that circle size may be taken in general to indicate, at least in a rough way, the measure of genetic affinity existing between the paired complexes which together make up the various species and hybrids. If this hypothesis is justifiable, then a new and very useful tool has been placed in our hands for the study of evolutionary trends within the genus. For, if two wild species are crossed and the F_1 shows a small circle, it would mean that the complexes contributed by the two species have a large proportion of their genes in common, that they have probably diverged relatively recently from a common source, and have not had time as yet to become greatly modified genetically; in other words, they are closely related. On the other hand, if two species are crossed and yield F_1 hybrids with a large circle, it would mean that the complexes contributed by the parents are distantly related. They have undergone a long evolutionary history since they first diverged from a common source. Thus, by crossing the various wild forms with one another, in all possible ways, and determining chromosome configurations of the F_1 hybrids, it ought to be possible to build up gradually an evolutionary tree of the *biennis* group of *Oenothera* which will probably be more accurate than family trees generally are, to say the least.

Incidentally another important result should accrue from such a procedure. It should be possible gradually to determine the arrangement of

ends of the various chromosomes in various forms, the essential first step in the construction of gene maps in the case of circle-bearing forms.

A beginning has already been made in a cytological study of wild forms and their hybrids. Sturtevant and Emerson are studying spontaneous forms from various parts of the country, and the present writer is engaged upon an analysis of wild California forms, with plans to extend this study to species growing in more and more outlying districts, should the study prove to be as valuable as it now promises.

Thus, the correlations between circle size and genetic relationships between complexes, which are apparently based upon the occurrence at rare intervals of segmental interchanges, may be the clues which will in time lead to a better understanding of the phylogenetic development of the *biennis* group of *Oenothera*.

Summary

Data are summarized which point to the existence of the following three correlations between chromosome behavior on the one hand and genetic relationship between complexes on the other:

1. Plants with identical genetic make-up have in general identical chromosome configurations in diakinesis.

2. Complexes are known which, although residing in different races, are nevertheless essentially identical genetically, or closely related. When essentially identical, such complexes give identical chromosome configurations when combined with a given third complex. When closely related, they give similar configurations when so combined.

3. Complexes which are closely related genetically yield, when combined with *each other*, configurations with most or all of the chromosomes paired. Those which are more distantly related give configurations in which more of the chromosomes are involved in circles.

It is suggested that these correlations are of widespread validity within the genus, and that they are to be explained on the basis of the segmental interchange theory. Assuming that such segmental interchanges as will produce viable forms have occurred with great infrequency, there has been ample time for gene changes to have occurred between successive interchanges, and consequently for the complexes making up wild forms to have become genetically divergent. As a result, circle size becomes, at least in a rough way, a measure of genetic similarity between complexes. Complexes which give smaller circles when combined are more closely related; those which give larger circles are more distantly related. This gives us a working tool for the analysis of relationships within the genus, and for a better understanding of the phylogeny of the group.

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THE VEGETATION OF A LAVA-FORMED LAKE IN THE CASCADE MOUNTAINS

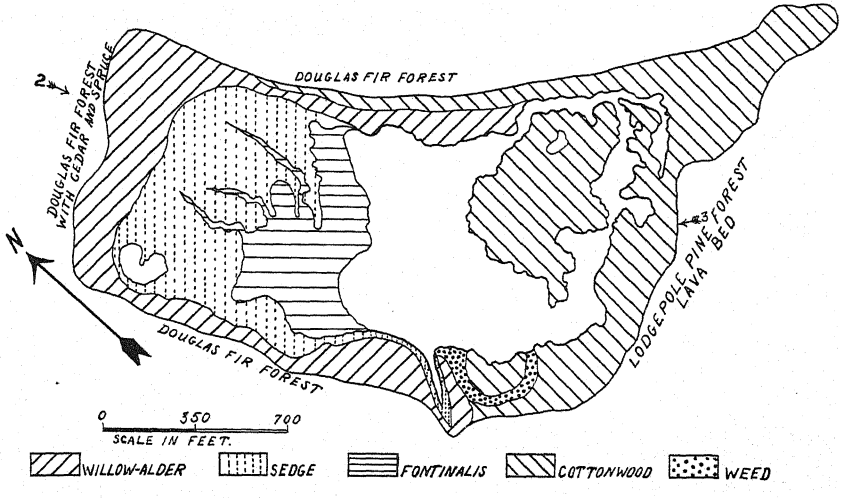
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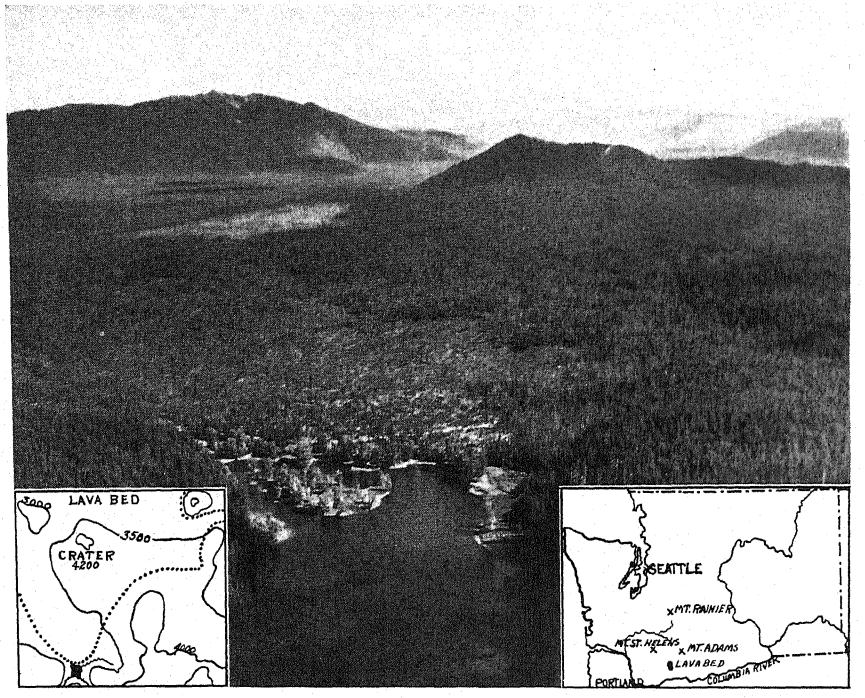
In the Cascade Mountains, lakes have occasionally been formed at relatively high elevations by the damming up of the head-waters of streams by lava flows sometime during past geologic ages (1, 2, 8). Such a lake is Goose Lake, located about twenty miles southwest of Mt. Adams on the Columbia National Forest in southwestern Washington at an elevation of about 3,000 feet.

During the recent geologic past a flow of lava issued, probably from a nearby cone now known as the Crater (4,200 feet elevation), filling to a varying depth the head of a mountain valley, and forming a bed about ten by five miles in extent. The lava dammed up many of the short creeks which drain the sides of the valley. The lava has numerous passages through it and most of these creeks disappear with hardly more than a temporary pond formed at the edge of the lava flow. A stream of considerable size drains out of the lower (south) end of the lava bed.

At the head (north) of the valley, several of these creeks concentrate their waters to form Goose Lake (text figs. 1 and 2). The lava extends part



TEXT FIG. 1. Sketch map of Goose Lake, drawn to scale from a vertical aerial photograph, showing vegetation zones and open water. Arrows 2 and 3 show the approximate directions from which the photographs in figures 2 and 3 were taken.



TEXT FIG. 2. Aërial oblique view of Goose Lake with the climax forest on either side and the Lava Bed beyond. Right insert shows location of the Lava Bed. Left insert is a map of a small portion of the Lava Bed (boundary line dotted) showing 500-foot contour lines. Map oriented the same way as the aërial photograph, showing Goose Lake in the foreground and the Crater in the middle distance. (Photo by Brubaker Aërial Surveys, Portland, Oregon.)

way under the lake, and in many places the bottom of the lake can be seen to be formed of lava, bare or covered with sediment. Only in the northwestern part of the lake, opposite the lava bed, is it likely that there is no underlying lava.

The lake is roughly rectangular in shape, with an area of about thirty acres. Near the southeastern corner is a low irregular island with an area of about seven acres. The southern shore of the lake slopes gently up to an abrupt, irregular jumble of rocks which marks the edge of the lava flow. The western and northeastern shores rise more abruptly and merge into steep mountain sides covered with the dense climax forest of the region. The northern shore slopes gently up into the valleys of two of the largest creeks which empty into the lake.

Fed by melting snows the creeks fill the lake to a high level in the spring. The water is constantly being drained from the lake through various "leaks" in the bottom and sides of the lake. Even as late as August the waters of the lake drain away through the largest of these subterranean passages

under the lava bed with a rushing sound. This subterranean drainage, the lake's only outlet, has a distinct effect on the water level of the lake, which at first is high and then slowly recedes as the amount of out-going water approaches and exceeds the amount of the gradually dwindling incoming water. The amount of water in the lake at the end of summer varies with the season. Of recent years an attempt has been made to locate and to dynamite these "leaks" and thus maintain the water level.

This attempt has been partially successful but there is still a considerable change in the water level which has a most interesting effect on the vegetation in the lake and along its shores. Using the water level as measured on July 5, 1929 [1] as the standard, the extreme high water level is nine feet above and the extreme low water level is five feet below this standard, making an annual fluctuation of about fourteen feet. Before the leaks were stopped the extreme low water level was fourteen feet below standard or an annual fluctuation of about twenty-three feet.

The average depth of the lake on July 5, 1929, was seven feet with the greatest depth twenty-two feet and the minimum depth three feet. The water is clear, very cold, and practically free from sediment except during the spring freshets. On July 5, 1929, the surface temperature was 60° F. and at the average depth (seven feet) 58° F. In the shallower places the water was about 63° F. but it becomes much warmer later in the season. The water of the inflowing streams was about 42° F. This temperature rises only a few degrees in August. A submarine spring in the upper end of the lake remains at about 52° F. throughout the summer.

Zones of Vegetation

A narrow zone along the southern, eastern, and northeastern shores is dominated by large, scattered cottonwoods (*Populus trichocarpa* Torr. & Gray) many of them two feet and more in diameter (text fig. 3). A very few conifers may be found scattered among them. Where the canopy of the cottonwood is more open, the undergrowth is dense and is composed of a variety of shrubs, herbs, and grasses. These shrubs are chiefly dogwood (*Cornus stolonifera* Michx.), *Spiraea douglasii* Hook., *Rubus parviflorus* Nutt., *Symphoricarpos albus pauciflorus* (Robbins) Blake, and *S. occidentalis* Hook. In wetter places dense thickets of *Salix sitchensis* Sans. with some *Lonicera involucrata* Banks may be found. Outward from this zone of cottonwood along the south shore is the sparse, open, lodgepole pine forest characteristic of the lava bed (text fig. 2).

Forming part of the south shore is an area of higher ground around which the water stands at extreme high water. This area is covered with cottonwood forming a dense canopy and supports only a sparse undergrowth of

[1] The data on depth, temperature, and depth of mud have been supplied by Mr. Jim Hutchings of Stabler, Washington, who has studied and measured the lake for many years and to whom my sincere thanks are due.

dogwood (*Cornus stolonifera* Michx.). The low ground around this higher area is covered, as the water recedes, with a growth of weeds which becomes very dense and three to four feet tall in late summer. These weeds are



TEXT FIG. 3. View of the shore vegetation and the island vegetation showing the climax forest on the hillside beyond. The cottonwoods are large and widely scattered with herbaceous and shrubby undergrowth (Photo by John T. Johnson).

chiefly *Artemisia tilesii* Ledeb. with a mixture of *Stachys ciliata* Dougl. and *Scrophularia occidentalis* (Rydb.) Bicknell. Nearer the edge of the lake there may also be a considerable growth of *Mentha canadensis* L. and *Radicula curvisiliqua* (Hook.) Greene.

On the comparatively level ground where two small intermittent streams flow in from the southwest there are thickets of willow (*Salix sitchensis* Sans.). These thickets are about ten feet high the stems sloping and criss-crossing in all directions, forming an all but impenetrable tangle. Mixed with the willow are a few specimens of dogwood, alder (*Alnus rubra* Borg.), and vine maple (*Acer circinatum* Pursh.). The undergrowth is sparse and is composed of *Urtica Lyallii* S. Wats., *Stachys ciliata* Dougl., and *Artemisia tilesii* Ledeb., named in order of their abundance.

Between these two small streams a bar of alluvium is formed which, even before the water level falls sufficiently to uncover it, is a solid mass of *Eleocharis palustris* L., and sterile shoots of *Equisetum arvense* L. This *Eleocharis* is in fact one of the most abundant species, covering extensive areas in the northern part of the lake (text fig. 1).

Near this alluvial bar is an extensive mass of lava rocks with considerable earth deposited in pockets and crevices. This supports a varied flora which develops as it is gradually exposed by the falling water level. Where the pockets of earth are deep and hold moisture longer, *Mentha canadensis* L. is found. In smaller crevices and pockets a number of species of *Carex* occur;

C. teneraeformis Mackenzie; *C. leporinella* Mackenzie; *C. exsiccata* Bailey; *C. athrostachya* Olney; *C. aperta* Boott, and *C. Kelloggii* W. Boott. *Juncus balticus* Willd. as well as several grasses; *Agrostis exarata* Trin., *Aira elongata* Hook., and *Aira caespitosa* L. are also found here. These sedges and grasses are the most abundant vegetation on this lava rock but there are also broad-leaved herbs, the most abundant of which are *Aster modestus* Lindl. and a form or variety of *Aster foliaceus* Lindl.

Along the west shore there are many springs and the whole hillside fairly oozes water. The cottonwood zone is absent but narrow zones of sedge and willow-alder are present. This hillside is covered with a fine virgin timber forest, the climax vegetation according to Piper (3) at this elevation, namely Douglas fir (*Pseudotsuga taxifolia* (Lam.) Britton), white pine (*Pinus monticola* D. Don), and white fir (*Abies* spp.). This forest supports a dense undergrowth typical of virgin timber in the Puget Sound region (3) with such mesic plants as devils club (*Echinopanax horridum* (Smith) Dcne. & Planch.), *Vancouveria hexandra* (Hook.) Morr. & Dec., *Tiarella unifoliata* Hook., *Cornus canadensis* L. and others. Ferns (*Dryopteris dryopteris* (L.) Christ. and *Athyrium filix-femina* (L.) Roth) are abundant as well as mosses and liverworts.

The northern part of the lake is a shallow expanse of water overlying a great depth of alluvial mud in which are growing submerged, great masses of *Fontinalis antipyretica gigantea* Sullivant. This water moss apparently grows much like *Sphagnum* as one can get partially decayed remains from considerable depths (four feet) below the surface of the mat of living plants. A pole can easily be pushed into this mass for many feet (average eleven feet), releasing great quantities of gas. Outward from this broad submerged zone is a very wide expanse of shallow water and mud flats covered with a dense growth of sedges, chiefly *Eleocharis palustris* L. This is followed by a wide zone of willow and alder and then the climax forest which here contains an admixture of western red cedar (*Thuja plicata* D. Don) and spruce (*Picea Engelmanni* Engelm.).

These sedge and willow-alder zones extend around to the northeastern shore. The sedge zone gradually narrows because of the steeper slope of the lake bottom until it disappears entirely, while the willow zone becomes narrowed to a mere fringe inward from the cottonwood zone but persists most of the way around to the eastern shore. Along the northeastern and eastern shore, as the water recedes we find inward from the willow zone a zone occupied by low sedges of various species and other herbaceous plants. The composition of this zone is variable but the most characteristic species is the small yellow-flowered creeping buttercup (*Ranunculus reptans* L.).

The island is covered by a sparse open growth of cottonwoods with a few conifers intermixed. There is a considerable shrubby and herbaceous undergrowth but the zones are for the most part indistinct. Some parts of the shore, however, show a distinct *Ranunculus* zone.

In the shallower, eastern portions of the lake extensive areas of the submerged *Persicaria hartwrightii* (A. Gray) Greene and *P. lapathifolia* (L.) S. F. Gray are found. These can be seen growing up from the bottom in early July. They reach the surface in late July and early August and soon send up their spikes of flowers. They make a very distinctive picture with their masses of pink flowers and their floating reddish tinged leaves. Curiously, these plants are not found in the western half of the lake. This may be due to the lower temperature of the water on this side from the cold inflowing streams (6), or more probably, the deep mud bottom and the sediment brought in by streams is detrimental to their growth. There are no other submerged or floating aquatics (aside from the *Fontinalis* already mentioned) found in the lake.

As the water level falls, more and more shore line is exposed. Late in the season, most of this shore line shows signs of plant growth even before it is fully exposed. The different species vary widely in their ability to grow while still submerged. The sedges and rushes grow extensively before being exposed while *Mentha canadensis* L., *Stachys ciliata* Dougl., *Radicula curvisiliqua* (Hook.) Greene, and *Ranunculus reptans* L. show less growth. On the other hand the grasses, *Aster foliaceus* Lindl., and *Artemisia tilesii* Ledeb. seldom make any signs of growth until fully exposed.

Discussion

The wide zone of *Fontinalis* is of interest because of its location in the lake. It seems, by its method of growth, to survive well the silting in of the lake at its upper end. If the lake is entirely underlain by lava, a layer of silt was necessary before any vegetation could take root. Investigators have shown that some silting seems necessary for the best growth and that silt determines the distribution of the aquatic vegetation. On the other hand too rapid silting in is detrimental to growth, either through its effect on turbidity of the water and consequent light intensity or through its actual smothering effect. In Goose Lake indications point to an annual deposition of 1/100 of an inch. How abundant *Fontinalis* is in other lakes of the region is not known nor do studies in near-by regions make any mention of it. Incidentally quantities of this moss have been used with indifferent success as a substitute for *Sphagnum* in the wrapping of forest nursery stock. It tends to mold easily and lacks the water retentiveness of *Sphagnum*.

Aside from the *Fontinalis* and *Polygonum* there are practically no free-floating or submerged aquatics. This may be due to the coldness of the water and the paucity of mineral nutrients (6). Aquatic vegetation seems to be represented in the Colorado (4) and central Sierra Nevada regions (7) by *Nymphaea polysepala* and *Sparganium augustifolium*.

The almost pure stand of *Eleocharis palustris* is paralleled by its similar behavior, as reported by Ramaley (5), in Colorado lakes where its effective-

ness in filling up the lake is pointed out. There is no indication of *Typha*, *Scirpus*, or *Phragmites*, common members of the plant communities of lakes at lower elevations. This may be due in part to their inability to withstand great changes in water level but it is more likely a matter of altitude as Ramaley (5) reports *Scirpus lacustris* present in the lakes of the plains and foothills areas but absent from the lakes of the montane zone and higher (6). In many lakes the sedge zone is formed of several species of *Carex* (4, 7).

The willow-alder zone is often made up wholly of willow near its inner edge, alder coming in on higher ground where this zone meets the forest. In Colorado lakes there is a willow-birch-honeysuckle zone. At Goose lake the last named species occurs only as scattered individuals in wet open places at the forests edge. The species of willow differ. Smiley (7) reports a willow zone with *Ranunculus* under it similar to the very indistinct zone of *Ranunculus reptans* inward from the willow as the water level lowers at Goose Lake.

No *Potentilla* zone is present nor are any species of *Potentilla* found around Goose Lake as occur around lakes in Colorado (4). The heath zone so common around alpine lakes in northeastern United States is lacking in the case of Goose Lake although it occurs to a limited extent around some small lakes located in open park-like forests at higher elevations in this region.

Summary

Goose Lake is a small lake in the Cascade Mountains of southwestern Washington at an elevation of 3,000 feet. It was formed by the damming up of several small creeks by a lava flow. Because of the porosity of the lava the waters of the lake constantly leak out through the sides and bottom of the lake causing a fairly regular annual fluctuation of the water level of about 14 feet.

The lake is about 1,000 by 2,000 feet in size. Silting and the decay of plants are gradually filling it up.

In the shallower deeply silted parts is a wide zone of the submerged water moss, *Fontinalis*, outward from which there is a wide zone of sedge, almost pure *Eleocharis palustris*. Next follows a zone of willow, *Salix sitchensis*, with some alder mixed with it at its outer edges. The drier southern shore of the lake has a zone of varying width of cottonwood, *Populus trichocarpa*, with a varied undergrowth of herbs and shrubs. In places a narrow indistinct zone of *Ranunculus* may be noted.

As the water level falls the newly exposed areas become covered with a variety of sedges, grasses, and herbs. These present an interesting study in their varied ability to start growth while still submerged.

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BEHAVIOR OF MOSAIC IN CERTAIN SUGARCANE VARIETIES IN LOUISIANA

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The disappearance of mosaic symptoms and the recovery of plants known to be affected are phases of the mosaic problem which have apparently received but scant attention in the past. It is generally assumed that plants once affected with a virus disease always remain affected and generally show a gradual deterioration or degeneration especially if propagated vegetatively. While such an assumption in the main may be well-founded, certain exceptions do occur which are of interest.

Of the exceptions which have been observed, it is interesting to note that a considerable number of them have been with the mosaic disease of sugarcane. This disease is peculiarly adapted for observations and studies of this nature. The sugarcane plant is propagated vegetatively, the disease being carried over from generation to generation in the stalks or buds, and the plant has a long period of growth, from nine months in Louisiana to as much as two years in the tropics. There are also a great many different varieties of sugarcane and these show all degrees of resistance from being practically immune to extremely susceptible. With such a plant any peculiarities in the behavior of a systemic disease will become noticeable at one time or another.

The possibility that mosaic-infected cane plants might be able to recover from the disease was suggested by Grey (8, 9) in 1919. From work carried on in Cuba, he claimed that mosaic symptoms would gradually disappear if the plants were fertilized and cared for properly. His claims were subsequently denied by Earle (2) and a controversy developed which failed to clarify the problem.

The disappearance of mosaic symptoms from the upper leaves of the partially resistant Badilla variety has been noted by Brandes (1) and observed by many other investigators in the tropics. In Hawaii, Lyon (1) has also observed a somewhat similar behavior of plants of the Lahaina variety. More recently Faris (7) has reported that in Cuba infected stalks of the P.O.J. varieties 2714, 2725, and 2727 often produce healthy plants.

INVESTIGATIONS IN LOUISIANA

At present in Louisiana, varieties of sugarcane are being grown which show all degrees of resistance and susceptibility to the mosaic disease. The varieties which are being grown commercially are largely canes of Java origin, known as P.O.J. canes. These are mostly hybrids between old standard

varieties and some of the wild oriental canes which are immune or highly resistant to the mosaic disease. A study of the behavior of the disease in these canes as well as in the older varieties has given some important information.

Work with Old Standard Varieties

In Louisiana, investigations on the mosaic disease of cane were started shortly after the disease was recognized in the state in 1919. At that time, the greater portion of the sugarcane acreage was planted to the varieties D 74 and Louisiana Purple. Both of these are very susceptible to the mosaic disease, and in fields in the older infected regions healthy plants are rare, though they do occur occasionally. It was noticed, however, that while the mosaic stripes on the leaves of most plants were ordinarily definite and distinct, stools with symptoms much less pronounced could be found in fields of both varieties. These stools were apparently more vigorous and the color of the leaves was of a darker green.

In 1920, stools showing the disease in a mild form were selected and planted. In the following year, canes in the selection plats showing the mildest mosaic symptoms were again used for planting. Since then, similar selections have been made each year. The results of this work (3, 4, 5, 6), indicate that at the present time these selected strains show the disease in an extremely mild form. In the spring, the mosaic symptoms show up faintly on the young shoots, but as the season advances, these gradually disappear. In November 1930 mosaic symptoms could not be found on a considerable portion of the plants in the selected plots. This is not entirely a masking of mosaic characters resulting from high temperatures or intense light conditions, as the plants in the unselected plots show the symptoms throughout the year. It is very evident that strains have been obtained which are extremely resistant or tolerant to the mosaic disease. How such strains can develop within a vegetatively propagated variety is not at present clear.

Work with P.O.J. Varieties

In the P.O.J. canes that are being grown in Louisiana, the behavior of mosaic is somewhat different from that in the old standard varieties. The four P.O.J. varieties upon which this paper is based, P.O.J. 36, P.O.J. 234, P.O.J. 213, and P.O.J. 228, are not immune to the mosaic, yet very rarely are fields found with all plants affected.

The P.O.J. varieties 36 and 234 are very susceptible to the disease and fields usually show a high percentage of infection during the growing season, frequently in the neighborhood of 90%. For several years, infection has remained about the same. In the most severely infected fields, it has always been possible to find plants free of the disease. The varieties P.O.J. 213 and P.O.J. 228, on the other hand, are more resistant to the disease than P.O.J. 36 and P.O.J. 234, and the infection in the field is usually light. In most fields of these varieties, the infection varies from a trace to five percent. An unusual infection of 40% was observed in one field of P.O.J. 213 at Re-

serve, Louisiana, in midsummer of 1930, yet on November 1 this had dropped to five percent. The reason for these low percentages, as will be brought out later in this paper, is due to the ability of the plants to throw off the disease or produce buds which are free of the virus.

Four Year Test with Mosaic-infected Stalks

An experiment to test out the behavior of mosaic in the P.O.J. canes has been in progress since 1926. In October of that year, twenty-five stalks of each of the varieties P.O.J. 213 and 228, which showed typical mosaic symptoms, were cut and planted. During the following year, the plants developing from these stalks were watched. Both varieties showed a fairly high percentage of mosaic during the summer, but during the fall there was a material decrease. In October (table 1), only six plants out of 366 of the variety P.O.J. 213 showed mosaic symptoms and 71 out of 221 plants of P.O.J. 228.

TABLE 1. *Mosaic Stalks Developing from Infected Seed Cane, 1927*

	5-7-27		8-15-27		10-19-27	
	Mosaic	Healthy	Mosaic	Healthy	Mosaic	Healthy
P.O.J. 213	28	54	177	136	6	360
P.O.J. 228	16	20	101	77	71	150

In the fall of 1927, the six mosaic stalks of P.O.J. 213 and a larger number of the infected stalks of P.O.J. 228 were planted and the progeny watched during the season of 1928. At the same time, a number of disease-free stalks from the same plat were planted. From the disease-free stalks, no mosaic plants developed. The results obtained from the diseased stalks are given in table 2. Again a considerable percentage of the plants developing from mosaic stalks were free of the disease.

TABLE 2. *Mosaic Stalks Developing from Infected Seed Cane, 1928*

	6-4-28		7-28-28		9-25-28	
	Mosaic	Healthy	Mosaic	Healthy	Mosaic	Healthy
P.O.J. 213	21	21	51	89	20	117
P.O.J. 228	170	214	287	412	326	372

In the fall of 1928, this experiment was continued by planting mosaic stalks from the plats of that year. In September 1929, counts were made of the number of mosaic and mosaic-free plants as in the previous year. In the P.O.J. 213 plat there were 98 diseased and 326 healthy plants, and in the P.O.J. 228 plat there were 121 diseased and 317 showing no mosaic symptoms. This experiment was also continued through 1929–1930 with results very similar to those obtained in the preceding years.

These tests demonstrated the fact that mosaic plants of P.O.J. 213 and 228 are constantly producing disease-free shoots even after being infected for four years.

Tests with Individual Stalks

In order to obtain more accurate information on the behavior of the mosaic in different cane varieties, a test was conducted so that the desired data could be obtained for each individual stalk. The varieties used in this test were L 511 and the P.O.J. varieties 36, 213, 228, and 234. L 511 is a cane that had been throwing off disease-free shoots from infected seed pieces for a number of years in a similar manner to the P.O.J. canes. In June 1929, fifty mosaic infected stalks from plats planted with mosaic infected cane the previous fall were selected and tagged. On October 18, these stalks were examined and the presence or absence of mosaic noted. The condition on that date is given in table 3. It will be seen that mosaic symptoms disappeared from some stalks of all varieties except L 511.

TABLE 3. *Mosaic Condition on October 18, 1929, of 50 Stalks Which Had Shown Mosaic Symptoms in June*

Variety	Number with Dead Tops	Number Showing Mosaic	Number Showing No Mosaic
P.O.J. 36	1	36	13
P.O.J. 213	4	33	13
P.O.J. 228	2	43	5
P.O.J. 234	9	36	5
L 511	8	42	0

On October 18, the fifty tagged stalks of each variety were cut and planted, each stalk being planted by itself and separated from the others by stakes. In late spring of 1930, the shoots developing from these stalks were examined for mosaic. The condition at that time is given in table 4. A comparison of the data in tables 3 and 4 will show that a number of stalks of P.O.J. 234 and P.O.J. 36, which were free of mosaic symptoms at the time of planting, produced diseased shoots, which suggests that masking may occur to some extent with these varieties. On the other hand, several stalks of both P.O.J. 213 and P.O.J. 228 which showed mosaic symptoms when planted, produced disease-free shoots.

TABLE 4. *Mosaic Condition on May 22, 1931, of Shoots Developing from 50 Stalks Known to have been Affected in June, 1929*

Variety	Number Stalks Producing Shoots	Number Showing Mosaic	Number Showing No Mosaic	Number Showing Both Mosaic and Healthy Shoots
P.O.J. 36	50	49	1	20
P.O.J. 213	47	27	20	17
P.O.J. 228	39	15	24	6
P.O.J. 234	45	43	2	4
L 511	25*	15	6	0

* The mosaic condition of four small plants was not determined.

In a second examination of these plats made on August 25, 1930, attention was paid to the location on the planted seed stalk of both the mosaic and mosaic-free shoots. From some stalks, only diseased shoots had been produced; from others, only healthy shoots, while some had produced both healthy and diseased shoots. The location of the healthy plants on the seed stalks, however, was not consistent. Several stalks of P.O.J. 213 and 228 produced mosaic-infected shoots from the basal half and healthy shoots from the top, while several others produced healthy shoots from the lower half and diseased shoots from the top. The mosaic condition of these plats is given in table 5.

TABLE 5. *Mosaic Condition on August 25, 1930, of Shoots Developing from 50 Stalks Known to Have Been Affected in June, 1929*

Variety	Number Stalks Producing Shoots	Number Stalks Producing Mosaic Free Shoots	Number Stalks Producing All Mosaic Infected Shoots	Number Stalks Producing Both Healthy and Mosaic Shoots	Number Stalks Producing Mosaic Shoots from Basal and not from Top Eyes	Number Stalks Producing Mosaic Shoots from Top and not from Basal Eyes
P.O.J. 36	50	0	21	29	7	1
P.O.J. 213	47	17	6	24	7	7
P.O.J. 228	39	21	5	13	5	2
P.O.J. 234	45	1	36	8	1	0
L 511....	25	2	20	3	1	1

Disappearance of Mosaic in Stubble (Ratoon) Cane

The plats from which the seed stalks used in the last described test were obtained were permitted to develop a stubble or ratoon crop in 1930. In June, 50 mosaic infected stalks of each of the four P.O.J. varieties were tagged. In October, 1930, these stalks were again examined and the presence or absence of mosaic determined. The results are given in table 6. With the exception of P.O.J. 213, there was less disappearance of mosaic than had been noted in previous tests. This was possibly due to the unusual growing conditions of 1930. Due to the drought in the spring and early summer, cane made most of its growth in the fall and was in a green, immature condition in late October.

TABLE 6. *Mosaic Condition in Late October, 1930, of 50 Stalks of Stubble Cane, Known to Have Been Affected in June*

Variety	Number Living	Number Showing Mosaic	Number Showing No Mosaic
P.O.J. 36	49	48	1
P.O.J. 213	45	26	19
P.O.J. 228	46	45	1
P.O.J. 234	47	42	5

Mosaic Behavior of Different Buds on Stalks

As it had been demonstrated that both healthy and diseased shoots may develop from an infected seed stalk, it seemed desirable to ascertain the location of the buds which produced healthy shoots. An experiment was started in the greenhouse in the fall of 1930, using the varieties P.O.J. 36, 213, 228, and 234. In Cuba, Faris (7) had conducted a somewhat similar test using P.O.J. varieties 2714, 2725, and 2727. He found that the mosaic disease was not present in all of the buds on the stalks. The varieties which he used are in Louisiana classed as immune, as mosaic has never been reported on them. They are certainly much more resistant than the varieties used in the Louisiana tests.

Twelve mosaic-infected stalks of each variety were planted in the greenhouse bench in October, 1930. On January 6, 1931, the shoots had reached a sufficient size to determine readily whether the mosaic was present. The stalks were removed from the ground and the behavior of the individual buds tabulated. In table 7, the results of the test are given.

TABLE 7. *Mosaic Condition of Shoots Developing from Mosaic-infected Stalks of Four Cane Varieties. Buds on the Stalk are Numbered from Bottom to Top*

Stalk	P.O.J. 36, Bud Number											
	1	2	3	4	5	6	7	8	9	10	11	12
1	0	0	0	0	−	+	+	0				
2	0	−	+	+	0	+	0					
3	0	0	0	−	0	−	−	0				
4	0	0	+	+	0	+	+	+				
5	0	0	0	0	0	−	+	0	+			
6	0	0	0	0	−	−	0	0				
7	0	0	0	+	+	0	+	+				
8	−	0	0	0	−	0	+	0	+	−		
9	0	0	0	0	+	+	+	+	0			
10	0	0	0	0	0	0	+	0	+	−	0	+
11	0	0	0	0	0	0	0	+	+	+		
12	0	0	0	0	0	0	−	0	−	0	+	

Stalk	P.O.J. 213, Bud Number										
	1	2	3	4	5	6	7	8	9	10	11
1	0	0	0	0	−	+	0	0	−	0	
2	0	0	0	0	0	0	+	+	+	0	
3	0	0	−	−	−	−	+	+			
4	0	−	0	0	−	0	−	0	0	0	+
5	−	0	0	0	0	−	−	−	−	+	
6	−	−	0	0	0	−	−	−	0	+	0
7	0	−	−	0	0	0	−	−	−	+	
8	0	0	0	0	0	0	−	−	+	+	0
9	0	+	0	0	0	−	+	0	+	+	
10	0	0	−	−	−	0	0	−	−		
11	0	−	0	0	0	0	−	−	−		
12	0	0	−	−	−	0	+	+			

Stalk	P.O.J. 234											
	1	2	3	4	5	6	7	8	9	10	11	12
1	0	0	0	0	0	+	+	+	0	+		
2	0	+	+	0	0	0	+	+	+	+		
3	0	0	+	+	+	0	0	+	+	+		
4	0	0	+	+	+	0	+	0	+	0		
5	0	0	+	+	+	+	0	0	0	+		
6	0	0	−	−	0	0	+	+	+	0		
7	0	0	0	+	+	+	−	−	0	0		
8	+	+	+	0	0	0	+	+				
9	0	0	0	−	−	−	0	0	+			
10	0	0	0	−	0	0	+	+	+	+		+
11	0	0	+	+	+	0	0	+	+	+		
12	0	0	+	+	+	+	0	0	+	+	+	

Stalk	P.O.J. 228										
	1	2	3	4	5	6	7	8	9	10	11
1	0	0	0	0	−	−	−	−	−		
2	0	0	0	−	−	−	−	−	+		
3	0	0	−	−	−	0	0	−	+		
4	0	0	−	−	−	0	0	−	−		
5	0	0	0	−	−	−	−	+	+		
6	0	0	−	−	0	0	−	+	0		
7	0	0	−	−	0	−	−	0	−		
8	0	0	−	−	0	+	0	−	−		
9	0	−	−	0	−	−	0	−	−	−	
10	0	0	0	+	+	0	+	0	+	+	
11	0	0	0	0	−	−	0	−	−	−	
12	0	0	0	−	−	−	0	−	0	+	

0 = no germination.
− = no mosaic symptoms.
+ = mosaic symptoms.

P.O.J. 36 and 234 are susceptible varieties yet there were a few scattered buds that produced healthy shoots. The resistant P.O.J. varieties, 213 and 228, on the other hand, produced a relatively high percentage of disease-free shoots. In this particular test, most of the shoots from the top part of the stalks of P.O.J. 213 and 228 showed mosaic, while the lower ones were mostly healthy. Whether this condition was more than a coincidence is not known as it does not seem to agree with the field results which had been obtained earlier. Possibly the results would have been slightly different if a greater germination of the basal eyes had been obtained.

Discussion

Since the mosaic disease of sugarcane was recognized in Louisiana in 1919, it has been possible to study the behavior of the disease in varieties showing varying degrees of resistance or tolerance. The varieties being grown commercially at that time, such as D 74 and Louisiana Purple, are susceptible to the disease, while the P.O.J. varieties which have been introduced since and have now become established in the state are usually considered as resistant or tolerant. The P.O.J. varieties 36, 213, 228, and 234 are hybrids between some of the old standard Java canes and certain wild canes from India known to be immune to the mosaic disease and have inherited to a greater or less degree the character of resistance. Of these four varieties, P.O.J. 36 and P.O.J. 234 are quite susceptible and fields usually show from 50 percent to 90 percent infection. On the other hand, it is unusual to see more than five percent infection in fields of P.O.J. 213 and P.O.J. 228. The behavior of the mosaic disease in these canes has been studied and some interesting variations have been observed.

The old varieties such as D 74 and Louisiana Purple do not commonly throw off the disease after they become infected. Cane is propagated vegetatively and the disease is carried through from one season to the next in the seed pieces or stubble. Certain exceptions, however, do occur with varieties of this type. The variety L 511 frequently produces healthy shoots from diseased seed pieces. Also in fields of Louisiana Striped or Ribbon cane, it is generally possible to find a few stools apparently free of the disease. Stalks from such stools are not immune to the disease as shoots developing from them are easily infected.

The noteworthy observation in regard to these varieties is that it is possible by selecting stools which show the disease in the mildest form to obtain gradually a strain which is practically free of mosaic symptoms. The plants are not free of the disease because under favorable conditions in the spring stripes can be seen on the leaves, but these largely disappear as the season advances. It is evident that the strains which have been obtained by selection are decidedly resistant to the disease. The explanation for this is not at present clear.

The behavior of the mosaic in the P.O.J. canes which have been studied

is somewhat different. These varieties when infected show the mosaic symptoms in as pronounced a form as many of the other varieties, yet the percentage of infection in the field has been much less. This has been found to be due to the ability of the canes to throw off the disease or to produce buds in which the virus is lacking. This may occur during the growing season but becomes more evident by planting stalks which are known to be infected. Buds from these stalks produce both infected and healthy shoots.

The so-called resistance of these varieties to the disease seems to be based on the ability of the variety to throw off the disease. If most of the buds on infected stalks produce disease-free shoots, the infection in the field remains low and the variety is said to be resistant. In this class are such varieties as P.O.J. 213 and P.O.J. 228. If only a small percentage of the buds produce disease-free shoots, the infection in the field will be fairly high, but all plants, will not be affected. Varieties in this class are P.O.J. 36 and P.O.J. 234. What is known then as resistance to the mosaic in cane varieties of this type is the ability of the plants to recover from the disease or to produce virus-free buds.

Summary

1. Cane varieties showing various degrees of resistance to the mosaic disease have been studied.

2. Certain of the old varieties such as D 74 and Louisiana Purple which were being grown at the time the disease was introduced into Louisiana do not to any great extent recover from the disease. In fields in the old infected areas, practically all plants show mosaic symptoms.

3. In varieties such as D 74 and Louisiana Purple, it has been possible gradually to select strains which show the mosaic symptoms in only the mildest form.

4. The P.O.J. canes which are hybrids between old standard varieties and wild immune canes, are not immune to the mosaic disease. Of the four varieties which have been studied in Louisiana, P.O.J. 36 and P.O.J. 234 are quite susceptible, while P.O.J. 213 and P.O.J. 228 are classed as resistant.

5. The relative resistance of these varieties is to a considerable extent due to the ability of the plants to throw off the disease and to produce buds which are free of the virus.

6. Infected stalks of these varieties, when planted, produce both healthy and diseased shoots. The resistant varieties, P.O.J. 213, and 228, produce a much greater number of healthy shoots than the susceptible varieties and this accounts for the relatively low infection in the field.

7. The disappearance of mosaic symptoms on the P.O.J. canes also commonly occurs in the field. Many plants showing the disease in early summer are apparently healthy in the fall. This is also more common with P.O.J. 213 and P.O.J. 228 than with P.O.J. 36 and P.O.J. 234.

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COMPARISON OF ROOT AND TOP DEVELOPMENT IN VARIETIES OF STRAWBERRY

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INTRODUCTION

While considerable work has been done in testing the productiveness, shipping qualities, disease resistance, quality, and other characters of strawberry varieties, very little study has been given to the root systems or to the relationship between the root system and the top. In the summer of 1929 the writer had the opportunity of making a comparative study of the root systems and tops of 18 varieties of strawberry grown under irrigation in the Horticultural Gardens of the Colorado Agricultural Experiment Station at Fort Collins.

Mann (1) described the origin, development, and function of the roots of the cultivated strawberry. As a new shoot or runner begins to grow, the adventitious roots develop very rapidly so that within about a month the young daughter plant may be transplanted. The crown of the young plant grows rapidly and additional adventitious roots form, usually in small groups. These roots are white in color, with few or no branches, and may reach a length of 20 cm. between the time of transplanting and the period of dormancy. Early the following spring the older parts of these roots turn from white to yellowish brown and numerous branches appear. A few new white roots also appear. After blooming and fruiting, browning and considerable decortication appear on the older parts, but the central core is healthy and lateral roots are vigorous. Additional new roots appear and follow the same cycle.

Weaver and Bruner (2) described the root system of a plant of the Dunlap variety growing in silt-loam soil near Lincoln, Nebraska. They found that plants of average size had about 45 roots per plant. The maximum depth and lateral spread were 37 and 12 inches, respectively. They report work showing that the Grand variety at Geneva, New York, had horizontal roots not over 6 inches long and that the maximum depth was 22 inches. They also report that the deepest roots of the Warfield variety at Madison, Wisconsin, penetrated scarcely down to two feet and that the lateral spread was hardly beyond the spread of the leaves.

MATERIALS AND METHODS

The garden in which the strawberry varieties were growing sloped just enough to the north to permit furrow irrigation. The surface soil was a

heavy clay-loam containing a small amount of sand. A few small stones were scattered throughout the soil. Earthworm holes were abundant in the layer between 3 and 8 inches in depth. The soil was very low in organic matter in spite of heavy applications of 15 to 20 tons per acre of barnyard manure that were made every 2 or 3 years. It contained so much clay that it dried slowly and warmed up slowly. On drying it formed irregular clumps that were very hard and compact.

The upper subsoil was a heavy clay, gradually becoming more sandy with increasing depth. Below 28 inches the sand content was pronounced. Drying caused the layer between 10 and 24 inches to form irregular masses. Calcareous masses were common below 21 inches.

The water-table was at a depth of about 16 feet. The surface soil drained well, so that water never stood on any part of the field. It dried slowly, however. At the time the roots were excavated, July 16, the soil was very moist, especially in the layer between 2 and 10 inches in depth, where it was of a putty-like consistency.

The varieties were planted during the latter half of April, 1928. Each variety, except the Beacon, Bliss, and Boquet, occupied one row 200 feet long. There were 150 plants set out in each row. The rows were 3.5 feet apart. Only 50 plants of each of the varieties Beacon, Bliss, and Boquet were set out. The Aroma, Champion, Eaton, Gibson, Howard 17, Marvel, Mastodon, and Pearl plants were secured from Michigan nurseries; the Chaska, Duluth, Easy Picker, Minnehaha, Nokomis, and Progressive plants from a Minnesota nursery; the Beacon, Belt, Bliss, and Boquet plants from New York nurseries; the Pride of Denver plants from a nursery at Boulder, Colorado; and the Oelrich plants from a grower at The Dalles, Oregon.

Each row of plants was irrigated immediately after planting in order to prevent any injury from drying. They were irrigated at intervals of 2 weeks during both summers. A mulch, 3 to 4 inches thick, consisting of manure and much straw was applied in early November, 1928. In early April, 1929, about one-half of this mulch was removed from the field and the remainder left to be cultivated into the soil to increase the humus content.

The area was irrigated about every two weeks. The surface was usually very dry before irrigations. In fact, most of the time the surface soil looked as though it had been allowed to become too dry. The six weeks preceding July 16, when the excavations were made, were very dry. The wet condition of the subsoil can be attributed, therefore, to the natural water-retaining power of the soil since it cannot be accounted for by excessive irrigation or by excessive rainfall.

The trench method of excavation was used. A trench about 2.5 feet wide and of sufficient depth to allow work on the deepest roots was dug across typical portions of the rows. Mother plants that were representative of the development of the particular variety were then selected from both sides of the trench. The root systems of two to four plants of each variety were then dug out in the usual manner by means of ice-picks.

Description of Roots and Tops

Aroma. The survival of plants in this variety was fairly good, being about 75 percent. The row, however, was very irregular in width. In a few places it was about 3 feet wide, but the average width was about 15 inches. The average height of the leaves was about 7 inches. There were few runners. A fairly large number of the leaves were yellow in color.

The plants excavated had 6 to 8 leaves, one fruiting stalk, and often one runner per plant. The crown was about one-half inch in diameter. The root system was characterized by a rather pronounced tendency to grow diagonally downward. The total number of roots per plant was 32 to 37 of which about one-third were dead. Many of the live roots were brown or black almost to their tips. The larger, healthier plants had a greater number of small, live, light-colored roots with well-developed branches than did the smaller plants. The maximum depth of the live roots was 10.5 inches but there was evidence of dead roots down to 16 inches. The working depth [1] was about 8 inches. A large number of roots spread laterally outward at a depth of 3 to 4 inches to a maximum spread of 24 inches. Lateral roots were usually not well developed.

The root system as a whole was not in a very thrifty condition as shown by the large proportion of dead roots, the rather poor growth of laterals, and the shallow depth. The shoot development was only fair.

William Belt. The row of this variety was very well developed. The survival was about 96 percent. The average width of the rows was fairly uniform averaging 24 inches. The average height of the leaves was 6 inches. Runners were few. The leaves were very yellow, only one variety, the Premier, exceeding it in yellowness.

The typical plants that were excavated had 5 leaves and one fruiting stalk per plant. The crown was about three-eighths of an inch in diameter. The root system in this variety was unusual in that practically all of the roots extended laterally or diagonally outward instead of going down below the plant. This left a block of soil directly below the crown which had very few, if any, roots in it. The total number of roots varied from 22 to 35, of which from one-third to nearly one-half were dead. They were mostly 0.25 to 0.5 mm. in diameter. There were very few new main roots and many of these were dead at the tip. The maximum depth of live roots was 15 inches with remains of dead roots down to 18 inches. The working depth was about 11.5 inches. The maximum spread was about 12 inches from the crown. Lateral roots were numerous and in good condition.

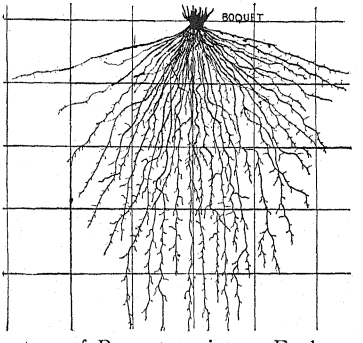
The root system as a whole was not in very thrifty condition as shown by the large number of dead roots and tips, but the top development was fairly good.

Bliss. The width of this row was fairly uniform, averaging 24 inches.

[1] The working depth means that depth to which many roots penetrate and much absorption must occur (2, p. 25).

The average height was 6 inches. The survival was about 90 percent. Runners were numerous. There was a moderate amount of yellowness in the leaves. The root system was not excavated.

Boquet (text fig. 1). This variety produced very poor growth. The row was very irregular and only 10 inches wide. The survival was only



TEXT FIG. 1. Root system of Boquet variety. Each square is 4 inches on a side. In each illustration the entire root system of a typical plant 15 months after transplanting is represented.

about 25 percent. The average height of the leaves was 5 inches. There were very few runners and the plants were moderately yellow in color.

The plants that were excavated had about 12 leaves and one fruiting stalk per plant. The crown was about one-half inch in diameter. The total number of main roots per plant was about 38 of which about one-third were dead. These roots were usually dark brown to black from the crown to the dead tip. Even the new main roots turned brown very rapidly. They varied from 0.25 to 1.25 mm. in diameter. The laterals which were fairly abundant were usually the only light-colored roots. No root hairs were seen on any of the roots. The cortex sloughed off very easily. The maximum and working depths were 20 and 16 inches, respectively. The maximum lateral spread was 12 inches.

As a whole the root system was not in a very thrifty condition as shown by the large number of dead or poor main roots, the dead tips and the lack of root hairs. The top development was poor.

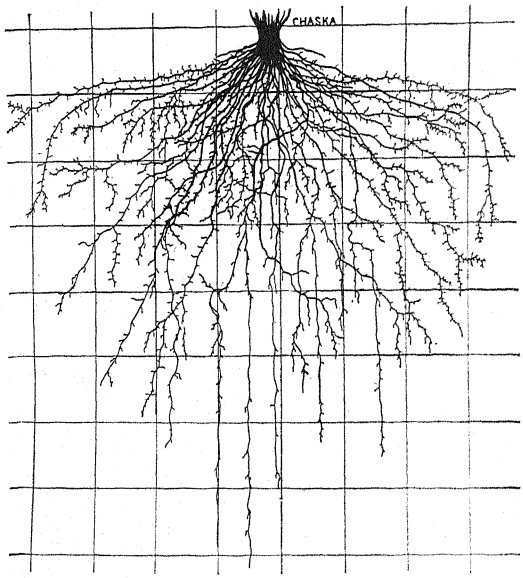
Champion. The row of this variety was dense and fairly uniform in width, averaging 28 inches. The average height of the leaves was 10 inches. The survival was about 100 percent. The runners were abundant. The leaves were rich green in color showing only a small amount of yellowness.

The plants excavated had about 24 leaves, 9 fruiting stalks, and 6 runners per plant. The total number of roots was about 70, of which only 3 percent were dead. The maximum depth of live roots was 28 inches, but evidence of dead roots was found down to 4 feet. The working depth was about 24 inches. The maximum spread was about 21 inches. Laterals were numerous, light-colored and healthy in appearance. Root hairs were evident

throughout. There was a large number of new, light-colored main roots. The older roots were usually dark brown in color. The spaces between the rows were well filled with roots.

The root system of this variety appeared very healthy as indicated by the large number of live main roots, the large number of thrifty laterals, the presence of root hairs throughout, and the low number of dead roots and tips. The depth and spread of the roots were also good. The top development was excellent.

Chaska (text fig. 2). The width of this row was very irregular, averaging 18 inches. The average height of the leaves was 5 inches. The survival was



TEXT FIG. 2. Root system of Chaska variety. Each square is 3 inches on a side.

60 percent. Usually there were few runners, but some of the plants had many. There was a moderate amount of yellowness.

The plants excavated had from 4 live leaves on the smaller plants to 18 on the large ones. There were 3 or 4 runners per plant and one fruiting stalk. The crown was from 0.5 to 1.0 inch in diameter. The total number of live main roots ranged from 29 to 43 of which 15 to 25 percent were dead. These roots were usually alive to the tip and the white or light color extended upward from the tip for considerable distances. The branching was well developed. The laterals were usually light-colored and appeared to be in very thrifty condition. The maximum and working depths were 24 and 18 inches, respectively. The maximum lateral spread was about 13 inches.

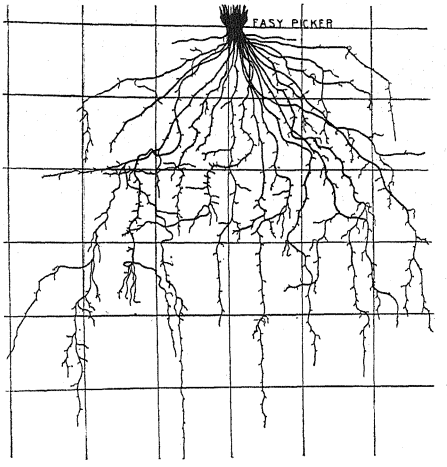
The root system of this variety was in good condition as indicated by the large number of thrifty, light-colored roots, small number of dead roots, well-developed branching, and the lack of dead tips.

Duluth. The average width of this row was 28 inches. It was fairly uniform and dense. The average height of the leaves was 8 inches. The survival was about 100 percent. Runners were very abundant. The amount of yellowness in the leaves was moderate.

The plants excavated had from 14 to 70 live leaves and 5 to 7 fruiting stalks per plant. The crown averaged 2 to 2.7 inches in diameter. The total number of main roots ranged from 75 to 100, of which 8 to 75 percent were dead. The maximum and working depths were 29 and 16 inches, respectively. The maximum lateral spread from the crown was about 40 inches. Most of the main roots spread out horizontally or diagonally downward so that there were very few roots directly under the crown. Lateral roots were numerous. The roots, especially the laterals, appeared healthier near the surface than deeper in the soil. Yellowish root hairs were found near the tips of some of the roots. Many of the root ends were dead, and usually only the tips were light-colored.

The depth, spread, number, and branching of the roots in this variety were very good. The large number of dead roots or tips on some plants and the presence of dead fungus growth on the main roots near the crown indicated that some of the plants were not very thrifty. The tops appeared to be in excellent condition.

Easy Picker (text fig. 3). The width of this row was very irregular, averaging about 20 inches. The survival was only about 60 percent. The



TEXT FIG. 3. Root system of Easy Picker variety. Each square is 4 inches on a side.

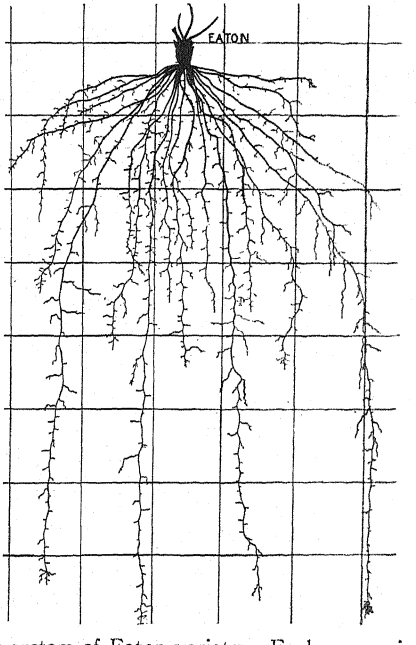
average height of the leaves was 5 inches. The number of runners varied from a few on some plants to many on others. The plants showed very little yellow coloration.

The plants excavated had from 4 to 7 leaves and one fruiting stalk per plant. The crown was about one-half inch in diameter. The total number

of roots varied from 20 to 30, of which about one-third were dead and about one-sixth were new. The lateral roots were rather few but in good condition. The root ends were usually very healthy, being white for a length of 3 to 5 inches. The root tips were usually alive and root hairs were abundant. The maximum and working depths were 24 and 15 inches, respectively. The maximum lateral spread was about 12 inches.

As a whole the root system was in good condition though not very extensive, while the tops were only fair.

Eaton (text fig. 4). The width of this row was very irregular, averaging 20 inches. The average height of the leaves was 7 inches. The survival



TEXT FIG. 4. Root system of Eaton variety. Each square is 3 inches on a side.

was good, averaging 80 percent. There was but little yellowness in the leaves. Usually there were few runners per plant, but a few plants had many.

The plants excavated had 3 to 5 live leaves and 2 or 3 runners per plant. The total number of main roots varied from 17 to 21 of which 50 to 65 percent were dead. Most of the live roots were black in color. The laterals were white and fairly numerous. There were a few root hairs on the white tips. The maximum and working depths were 24 and 15 inches, respectively. The maximum lateral spread was 11 inches.

The general development of the root system was rather poor and that of the tops fair.

Gibson. Only about 50 percent of the original plantings had survived.

There were few runners. The width of the row was very irregular, averaging about 20 inches. The average height of the leaves was 7 inches. There was considerable yellowness in the leaves.

The plants that were excavated had from 11 to 17 live leaves, one or two runners, and one fruiting stalk per plant. The crown was about 1.2 inches in diameter.

A large number of roots radiated downward and obliquely outward from the well-developed crown. The total number of roots per plant varied from 50 to 71, of which 15 to 40 percent were dead. They varied from 0.25 to 1.0 mm. in diameter.

The root system was very shallow. The maximum depth was only 12 inches and the lateral spread from the crown 13 inches. At the maximum lateral spread the depth was 10 inches. The working depth was about 8 inches.

Although many of the roots were dead at the tip the root system as a whole was in fairly thrifty condition as indicated by the presence of a few new main roots and many new laterals. It appeared that these new roots were probably taking the place of those that had died. The general development of the tops was poor.

Howard 17 (Premier). The row of this variety was fairly uniform in width, averaging 10 inches. The average height of the leaves was 5 inches. The survival was fair, averaging 70 percent. Runners were few. The plants were very yellow, being the most yellow of all the varieties.

The plants varied much in size. The number of live leaves per plant ranged from 8 to 25. There were usually 2 fruiting stalks per plant. The diameter of the crown varied from a half-inch to 2 inches. The total number of live main roots ranged from 27 to 38 of which 25 to 33 percent were dead. These main roots were usually dark brown in color from the crown to close to the tip. Even the new roots, which were fairly numerous, turned black and shriveled very rapidly. It appeared that many roots were dying. Laterals and secondary laterals were well-developed. The maximum and working depths were 15 and 12 inches, respectively. The maximum lateral spread from the crown was 18 inches.

As a whole this root system was not in a very thrifty condition as indicated by the large number of dead and dying roots and by the rapid rate at which new roots turned black and shriveled. The production of rather numerous, well-branched long laterals may have been related to the death of the tips of the main roots. The general development of the tops was fair.

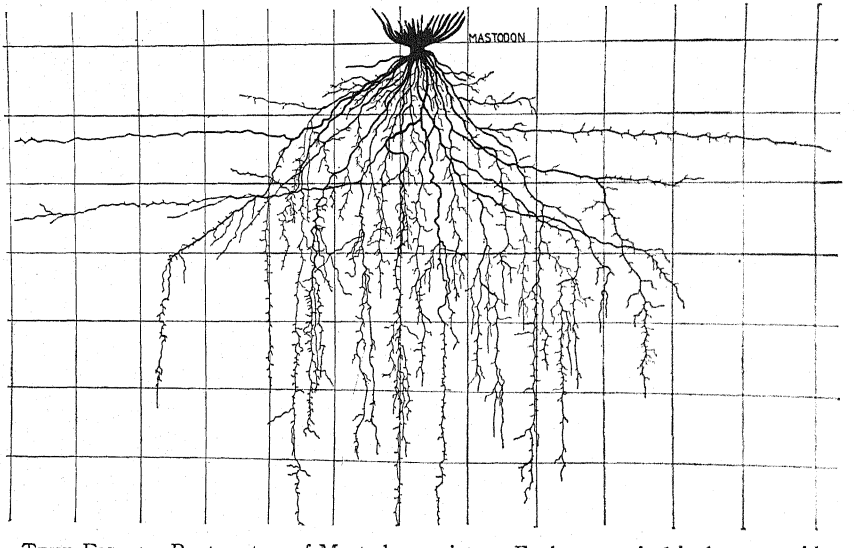
Marvel. The row of this variety was well developed. It was fairly uniform in width, averaging 36 inches, and the plants in the row were dense. The survival was about 100 percent. The average height of the leaves was 10 inches and runners were very abundant. There was very little yellow coloration.

The plants excavated had 8 to 18 leaves, 2 to 5 runners and one fruiting

stalk per plant. The total number of roots per plant varied from 19 to 30, of which about one-third were dead. About one-fourth of the roots were new. The crown was about three-eighths inch in diameter. Laterals were fairly abundant and in good condition. The maximum depth was about 17 inches and the working depth about 13. The maximum lateral spread from the crown was 14 inches.

The root system as a whole was in a thrifty condition as indicated by the light color and the live tips of many of the main roots. The general condition of the tops was good.

Mastodon (text fig. 5). This variety produced excellent growth. The row was very dense and uniform in width. It averaged 28 inches wide.



TEXT FIG. 5. Root system of Mastodon variety. Each square is 6 inches on a side.

The average height was 10 inches. The survival was about 100 percent. Runners were fairly numerous. There was, however, considerable yellow coloration.

The plants excavated had 24 to 36 leaves and 5 fruiting stalks per plant. The crown averaged 1.75 inches in diameter. The total number of live main roots ranged from 35 to 40, of which 8 to 30 percent were dead. The diameter of a root near the crown was usually about 1 mm. The maximum depth of live roots was 40 inches, but evidence of dead roots was found down to 48 inches. The working depth was about 30 inches. The maximum lateral spread was 36 inches. Laterals were numerous and many of them were 3 to 12 inches long. Nearly all of the roots were in excellent condition. They were usually alive to the tip and new main roots were fairly numerous. About one-half of the distal ends of the main roots were white or light brown in color.

The root system of this variety was in excellent condition as indicated by the depth and spread of the roots, the large development of laterals, and the healthy, light-colored appearance of most of the roots. A white mold, however, was rather abundant on the older main roots. The condition of the tops was also excellent.

Minnehaha. The width of this row was very irregular, averaging only 10 inches. The average height of the leaves was 5 inches. The survival was poor, being only about 50 percent. There were few runners. The plants were only slightly yellow.

The plant excavated had 12 leaves, one fruiting stalk, and one runner. The double-headed crown was about 1.25 inches in diameter. There was a total of about 100 main roots, of which 25 percent were dead. The diameter of the root averaged from about 1.5 mm. near the crown to 0.5 mm. at 6 inches. Most of the live roots were dark brown in color. The dark color persisted to near the root-tip which was often dead. Curious swellings, two to three times the normal diameter, were found rather frequently at depths of 9 and 10 inches on the main roots. Laterals were numerous, often 6 to 8 per inch of main root. Root hairs were well-developed, often forming a brush-like cluster at the root tips. Frequently near the root tip a cluster of 8 laterals per inch was found. The development of the large number of laterals and laterals of the second order may have been due to the death of the tip of the main root. The maximum and working depths were 16 and 14 inches, respectively. The maximum spread was about 12 inches.

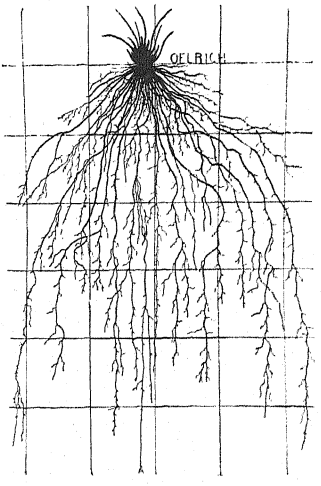
This variety had a good root system except for the rather large number of dead roots and shallow depth of penetration. The large number of thrifty laterals and root hairs were indicative of an efficient root system. The tops were very poorly developed.

Nokomis. The row of this variety was very irregular in width, averaging 22 inches. The survival was only 50 percent. The average height of the leaves was 8 inches. Usually there were few runners, but a few plants had many. There was a moderate number of yellow leaves.

The plants excavated had only 2 to 3 live leaves and one fruiting stalk per plant. The crowns were usually about three-eighths of an inch in diameter, but the occasional double-headed crowns were over an inch in diameter. The roots varied from 42 on smaller plants to 70 on the larger ones. About 75 percent of the roots were dead. The live roots were usually dark brown or black in color and dead at the tips. Laterals were fairly numerous, often 4 to 8 per inch of main root. The maximum depth of the live roots was about 10.5 inches but dead roots were found down to 16 inches. The working depth was about 10 inches. The maximum lateral spread from the crown was about 16 inches.

As a whole the root system appeared decidedly unhealthy as shown by the large number of dead roots and the dark color and dead tips of the live roots. The top development was also poor.

Oelrich (text fig. 6). The row of this variety was very irregular in width, averaging about 18 inches. The average height of the leaves was 5 inches. The survival averaged only about 40 percent. The runners, however, were fairly numerous and the plants were only slightly yellow in color.



TEXT FIG. 6. Root system of Oelrich variety. Each square is 4 inches on a side.

The plants that were excavated had 16 leaves, 2 to 5 runners, and one fruiting stalk per plant. The diameter of the crown was 0.75 to 1.0 inch. The total number of roots per plant ranged from 34 to 40, of which from 20 to 33 percent were dead. The diameter of the root averaged about 0.5 mm. The maximum depth was 24 inches, the working depth about 16 inches. The maximum spread was 12 inches. Laterals and secondary laterals were well-developed. There were not many new roots and the color of the roots was usually dark brown to black. The root tips were usually alive.

As a whole the root system was in good condition as indicated by the live root tips and well-developed branching, but the tops were poorly developed.

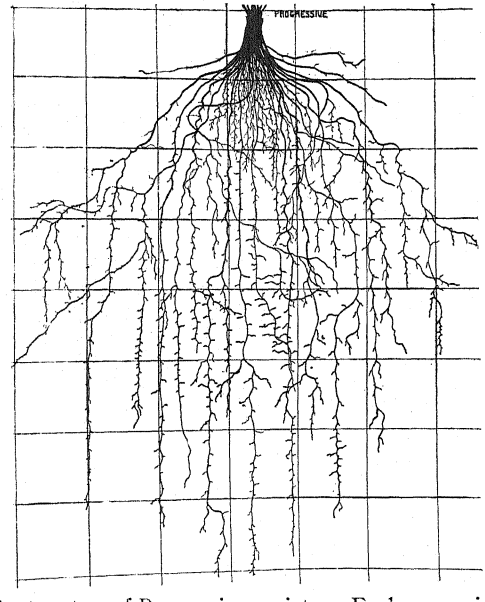
Pearl. The development of the row of this variety was very poor. The survival was only about 15 percent. The average width of the row was rather uniformly about 5 inches and the average height of the leaves 4 inches. Usually there were no runners. There was considerable yellowness in the leaves.

The root system and tops of this variety were very poorly developed. The typical plant that was studied in detail had only 8 live roots and 47 dead ones, 0.5 to 1.0 mm. in diameter. The crown was about 1.5 inches in diameter. This plant had about 13 live leaves and one fruiting stalk. Live lateral roots were rather numerous on the 8 main roots, due perhaps to the death of many main roots. The maximum depth of the live roots was about 12 inches but there was evidence of dead ones down to 28 inches. The

working depth was about 8 inches. The maximum spread from the crown was about 14 inches and the greatest depth at this distance was about 2 inches. The tips of many of the main and lateral roots were dead. Secondary and tertiary laterals were fairly well-developed on the live roots. It appeared that the plant was endeavoring to remain alive by replacing dead roots with new main roots which were often well-branched.

Pride of Denver. This variety produced poor growth. The survival was only about 25 percent. The row was very irregular, averaging about 15 inches in width. The height of the leaves averaged 6 inches. There were very few runners and most of the plants were very yellow.

The plants excavated had 9 to 12 live leaves and up to 2 runners per plant. The diameter of the crown was about one-half inch. There were from 20 to 30 roots, of which about one-fifth were dead. Most of them were about 0.5 mm. in diameter. The maximum depth was 12 inches and the working depth 8 inches. The maximum lateral spread was 16 inches.



TEXT FIG. 7. Root system of Progressive variety. Each square is 3 inches on a side.

Although shallow the root system was in a thrifty condition as shown by the healthy, light-brown color and white tips of the new main roots, by the fairly numerous light-colored laterals and by the scarcity of dead roots. A striking characteristic of the root system of some of the young plants was the thickness of the main roots and the enlarged tips. The condition of the tops was very poor.

Progressive (text fig. 7). The row of this variety was somewhat irregular in width but the density in the row was very uniform. The width averaged

TABLE 1. *Comparative Development of Root Systems and Tops of 18 Varieties of Strawberry*

	Depths of Root System, inches		Maximum Lateral Spread, inches	Ave. No. Live Roots per Plant	General Condition of Root System	Ave. Width of Top inches	Ave. Height of Top inches	Survival of Original Plantings, percent	No. of Runners	Yellowness	General Condition of Tops
	Maximum	Working									
Aroma	10.5	8	24	23–25	poor	15	7	75	few	moderate	fair
Beacon	—	—	—	—	—	24	7	96	many	moderate	very good
Belt	15	11.5	12	15–18	poor	24	6	96	few	much	good
Bliss	—	—	—	—	—	24	6	90	many	moderate	good
Boquet	20	16	12	26	poor	10	5	25	few	moderate	poor
Champion*	28	24	21	68	excellent	28	10	100	many	little	excellent
Chaska	24	18	13	25–33	excellent	18	5	60	few	moderate	fair
Duluth*	29	16	40	25–70	very good	28	8	100	many	moderate	excellent
Easy Picker	24	15	12	14–20	good	20	5	60	moderate	little	fair
Eaton	24	15	11	7–9	poor	20	7	80	few	little	fair
Gibson	12	8	13	42	fair	20	7	50	few	much	poor
Howard 17 (Premier)	15	12	18	20–26	fair	10	5	70	few	very much	poor
Marvel	17	13	14	13–20	good	36	10	100	many	little	very good
Mastodon*	40	30	36	28–33	excellent	28	10	100	many	much	excellent
Minnehaha	16	14	12	75	fair	10	5	50	few	little	very poor
Nokomis	16	10	16	11–18	poor	22	8	50	few	moderate	poor
Oelrich	24	16	12	28	good	18	5	40	many	little	poor
Pearl	12	8	14	8	poor	5	4	15	very few	moderate	very poor
Pride of Denver*	12	8	16	16–24	fair	15	6	25	very few	much	very poor
Progressive*	24	20	12	14–43	very good	22	7	90	few	little	good

* So-called "everbearing" varieties.

22 inches. The average height of the leaves was 7 inches. The survival was 90 percent. The leaves usually had a good green color, only a few leaves being yellow. Usually there were only a few runners, but a few plants had many.

The plants excavated had from 10 to 25 live leaves, 1 to 4 fruiting stalks, and 2 to 5 runners per plant. The crowns ranged from 0.4 to 1.5 inches in diameter. The number of main roots varied from 18 to 58 per plant, of which 8 to 25 percent were dead. New, light-colored roots were fairly numerous. Many of the roots pursued a very sinuous or tortuous course. The irregularity of branching heightened this characteristic appearance. The maximum and working depths were 24 and 20 inches, respectively. The maximum lateral spread was about 12 inches. Laterals were fairly numerous and long. Most of the laterals and secondary laterals were light-colored and appeared very healthy.

The root system as a whole was in good condition as indicated by the large proportion of live main roots, live tips, and the well-developed healthy laterals. The condition of the tops was also good.

Discussion of Relationship Between Root and Top Development

Data are summarized in table 1 showing the comparative development of both tops and root systems of the different varieties.

The best developed root systems were found in Champion, Chaska, Duluth, Mastodon, and Progressive. All of these are everbearers except Chaska. The best development of tops was found in Champion, Duluth, Marvel, and Mastodon. The poorest root systems were in Aroma, Belt, Boquet, Eaton, Nokomis, and Pearl. The poorest tops were in Boquet, Gibson, Howard 17, Nokomis, Oelrich, Pearl, and Pride of Denver. Fair to good root systems were found in Easy Picker, Gibson, Howard 17, Marvel, Minnehaha, Oelrich, and Pride of Denver. Fair to good tops were found in Aroma, Belt, Bliss, Chaska, Easy Picker, Eaton, and Progressive.

Deepest root systems (maximum depths of 28 to 40 inches) were found in Mastodon, Duluth, and Champion. The following varieties had moderately deep roots (maximum depths of 16-24 inches): Boquet, Chaska, Easy Picker, Eaton, Marvel, Minnehaha, Oelrich, and Progressive. Shallow root systems (maximum depths of 15 inches or below) were found in Aroma, Belt, Gibson, Howard 17, Nokomis, Pearl, and Pride of Denver.

Most of the varieties had some yellow color in the leaves. Those showing most yellow were in order: Howard 17, William Belt, Pride of Denver, and Mastodon. Those showing the least yellow were: Champion, Easy Picker, Eaton, Progressive, Marvel, Minnehaha, and Oelrich.

As a rule there was more or less relationship between top and root development. Varieties with poor top development usually had poor to fair root systems. The root systems may have been poor due to shallowness, limited spread, large percentage of dead roots, dead tips, lack of branching

and root hairs, and lack of thrifty roots as indicated by the light color. The everbearers, Champion, Duluth, Mastodon, and Progressive usually had good tops and good root systems. Pride of Denver, Pearl, Minnehaha, Aroma, Boquet, Eaton, Gibson, and Howard 17 had poor to fair tops and root systems.

In a few varieties there was little or no relationship between top and root development. In Chaska the root system was excellent and the tops fair. In Oelrich the root system was good and the tops poor. In Minnehaha, the root system was fair and the tops very poor.

Best development in the width of rows (24 to 36 inches) was found in Belt, Bliss, Champion, Duluth, Marvel, and Mastodon. Poorest development (5 to 15 inches) occurred in Aroma, Boquet, Howard 17, Minnehaha, Pearl, and Pride of Denver.

Greatest survival (90 to 100 percent) of original plantings occurred in Belt, Bliss, Champion, Duluth, Marvel, Mastodon, and Progressive. Poorest survivals (15 to 50 percent) were found in Boquet, Gibson, Minnehaha, Nokomis, Oelrich, Pearl, and Pride of Denver. Differences in survival may have been due partly to the condition of the original plants since some varieties came from greater distances than others.

Summary

1. The root systems and tops of 18 varieties of strawberry were studied at Fort Collins, Colorado. Soil and cultural methods are described in detail.

2. The root systems under these conditions were usually shallow. The maximum depth ranged from 10.5 inches in Aroma to 40 inches in Mastodon. The working depth varied from 8 inches in Aroma, Gibson, Pearl, and Pride of Denver to 30 inches in Mastodon. The maximum lateral spread from the crown varied from 12 to 40 inches. The main roots were usually about 1 mm. in diameter near the crown. The root color was usually brown to black except in young, vigorous roots where it was white or yellowish. Healthiness in the root systems was indicated by number of live roots, which varied from 7 in Eaton to 75 in Minnehaha; by vigorous appearance; by abundance of laterals and root hairs, and by the depth and spread.

3. A number of vegetative characters indicated the comparative development of the tops. The average width of the rows varied from 5 inches in Pearl to 36 inches in Marvel. The average height of the leaves ranged from 4 inches in Pearl to 10 inches in Champion, Marvel, and Mastodon. The survival of original plantings varied from 15 percent in Pearl to 100 percent in Champion, Duluth, Marvel, and Mastodon. The number of runners was usually few, but in a few varieties, as Champion, they were very abundant. The varieties differed a great deal in the degree of yellow coloration of the tops. The yellowest ones were Belt, Gibson, Howard 17, Pride of Denver, and Mastodon. The least yellow were Champion, Easy Picker, Eaton, Marvel, Minnehaha, Oelrich, and Progressive.

4. The varieties may be grouped into three classes according to the development of the root systems. The varieties with excellent or very good root systems were Champion, Duluth, Mastodon, Progressive, and Chaska. The first four are everbearing varieties. Those with fair to good root systems were Easy Picker, Gibson, Howard 17, Marvel, Minnehaha, Oelrich, and Pride of Denver. Only the last is an everbearing variety. Varieties with poor root systems were Aroma, Belt, Boquet, Eaton, Nokomis, and Pearl.

5. The varieties may also be grouped into three classes according to development of the tops. Those with very good or excellent tops were Champion, Duluth, Marvel, and Mastodon. All of these except Marvel are everbearers. Those with fair to good tops were Aroma, Belt, Chaska, Easy Picker, Eaton, and Progressive. Only Progressive is an everbearer. Those with poor or very poor tops were Boquet, Gibson, Howard 17, Minnehaha, Nokomis, Oelrich, Pearl, and Pride of Denver. Only the last is an everbearer.

6. Considerable relationship was observed between the development of root systems and tops. The varieties with very good to excellent development of both tops and root systems were Marvel and the everbearing varieties Champion, Duluth, Mastodon, and Progressive. Those with poor to fair development of both tops and root systems were Aroma, Boquet, Eaton, Gibson, Howard 17, Minnehaha, Nokomis, Pearl, and Pride of Denver. A few varieties showed little or no relationship of development between tops and roots. In Belt the root system was poor and the tops fairly good; in Chaska the root system was excellent and the tops fair; in Oelrich the root system was fairly good and the tops poor.

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POLYEMBRYONY AND GERMINATION OF POLYEMBRYONIC CONIFEROUS SEEDS

TEMA SHULTS CLARE AND GEORGE R. JOHNSTONE

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The morphological investigations dealing with embryogeny and polyembryony in small-seeded conifers according to Buchholz (1, 5) have not been accompanied by any observations of the germination of polyembryonic seeds. Such observations, reported here, have been made incidental to certain germination studies dealing primarily with chilling as a factor in hastening the germination of five large-seeded species: *Pinus torreyana* (Torrey pine), *Pinus sabiniana* (digger pine), *Pinus cembroides* var. *monophylla* (one-leaf piñon), *Pinus Coulteri* (Coulter pine), and *Pinus tuberculata* (knob-cone pine). In addition, 50 seeds of each species have been dissected to determine the nature and relative positions of their embryos; while the degree of the elimination of the endosperms from the mature seeds of *Pinus torreyana*, *Pinus cembroides* var. *monophylla*, and *Pinus banksiana* has been determined by weighing the endosperms and embryos intact, and separately. Apparently, the literature reporting the observations of the germination of polyembryonic conifer seeds is brief.

The occurrence of two well developed seedlings from the same seed seems to be very rare in pines. Toumey (10) reported that the extensive germination tests on a large number of indigenous and exotic conifers which had been conducted over a period of twenty years at the Yale School of Forestry showed in one instance two well developed and normal seedlings from one seed of *Pinus thunbergii*. *Pinus lambertiana* (sugar pine) is more prolific. Jacobs (6) found, while experimenting with the germination of sugar pine seeds, that twelve out of as many hundred developed two young seedlings each. The significance of the occurrence of such twin seedlings is difficult to determine.

The importance of embryogeny, and the origin and significance of polyembryony, have been pointed out by Buchholz (2, 5), who stated that embryogeny must occupy a much more important place in the comparative morphology of the conifers than has generally been conceded, and that simple polyembryony (2) due to the plurality of archegonia and arising from the fertilization of two or three eggs is general, but the complete development of more than one embryo in a mature seed is seldom found. Furthermore, cleavage polyembryony (4), the separation of the zygote into smaller units, often occurs. These units undergo competition with each other. Morphological evidence (4) is presented to support the hypothesis that most of the

living conifers whose embryogenies are known, perhaps all of them, have passed through a stage of cleavage polyembryony in their evolution. Cleavage polyembryony is therefore a primitive feature so far as conifers are concerned. Mottier (8) agrees with Buchholz concerning polyembryony in the genus *Pinus* resulting from cleavage of the proembryo into four embryos. All of these tend to develop but, owing to competition, selection results and only one embryo matures in a seed. Usually the embryo plunged more deeply chalazalward in the endosperm is largest and finally matures.

The purpose of the following investigation was threefold: first, to observe the occurrence of two well developed seedlings from a single seed to show the degree of rarity of this phenomenon; second, to dissect the seeds to show the nature of the embryos; and third, to weigh the endosperms and the embryos intact and separated to indicate the degree of the elimination of the endosperms.

Materials and Methods

The seeds which are used in the following experiments were obtained from three sources. Torrey pine seeds of the 1928 crop were purchased March 11, 1929, from Mr. Hartman, La Jolla, California; while the seeds of digger pine, one-leaf piñon, Coulter pine, and knob-cone pine from the 1929 crop were obtained through the courtesy of Mr. Bodine of the Los Angeles County Forestry Department, Henninger Flats Reforestation Reserve, and the seeds of *Pinus banksiana* were contributed by Raphael Zon of the Lake States Forest Experiment Station. These seeds were collected from the Chippewa National Forest, Minnesota, in November 1929, and tested at the Forestry Station, giving 59 percent germination in 33 days.

Seed treatment included storage, selection, chilling on ice, and planting in sheet iron trays containing sandy loam soil. The seeds were stored in paper bags at room temperature until they were prepared for planting. This preparation began when the seeds were placed in tap water to select those of better quality which were the heavier seeds that sank to the bottom. Such seeds were used in the germination tests for all species excepting *Pinus tuberculata* whose seeds could not be thus segregated. In the germination tests all seeds were soaked in running water for twenty-four hours before chilling or planting unchilled as controls. The seeds, whether chilled or unchilled, were planted in lots of 10, a number too small for satisfactory results, but seeds were scarce and difficult to obtain in large quantities. However, each experiment was repeated. The chilling of a sufficiently large number of seeds to provide several lots of ten each was obtained by placing the seeds in cloths well spread out on ice. The seeds and ice were then enclosed by wrapping them with heavy paper. The chilled seeds were removed from the ice in lots of 10 at five day intervals and placed to germinate in moist soil at room temperature ranging from 23° to 25° C. Evidence of polyembryony was observed by examination of the seedlings. This method might lead to error unless one is able to present evidence that the

multiple seedlings come from the same ovule. This source of error is not likely to occur in pine seeds if they are spaced properly when planted.

The dissection of the seeds was planned to show the nature and the origin of the multiple embryos. Fifty seeds of each species were dissected using a sharp scalpel, a pair of fine pointed forceps, and dissecting needles.

The weighing method was employed to determine the degree of elimination of the endosperms from three species: *Pinus torreyana*, *Pinus cembroides* var. *monophylla*, and *Pinus banksiana*. Accordingly, the testas of 60 seeds of each species were removed and the endosperms containing the embryos were weighed. The embryos were then removed from the endosperms, and the embryos and endosperms were weighed separately. In addition to expressing the results in grams a quotient was determined by dividing the weight of the embryos by the weight of the endosperms.

Results of Germination

Pinus torreyana

Polyembryonic seeds may germinate, producing multiple seedlings. Of the 173 Torrey pine seeds which germinated there were two instances of two well developed seedlings from single seeds. Figure 1, Plate XLVI, shows two such seedlings as they appeared when removed from the soil. One-half of the seed coat and a small portion of the endosperm were present at the division between the stems and the roots; while, in the majority of cases where only one seedling developed from a single seed, as shown in figure 2, the seed coat and the remaining portion of the endosperm were raised above the ground level on the cotyledons. The comparative size of the two seedlings of Torrey pine and their relative positions are also shown in figure 1. The top of one was pushed to the left, however, to show more of the seed coat. This seedling had the larger root which coiled around within the seed coat and then grew downward into the soil. The exact dimensions of these seedlings are as follows: the stem of the one with the straight root is $5\frac{1}{2}$ cm. long and its root is 4 cm. in length, while the stem of the other seedling is 4 cm. long and its root is 10 cm. in length. Each seedling has 10 cotyledons; however, the one with the shorter stem had five cotyledons of normal size on one side and on the other side the five were about one-half the length of normal cotyledons.

The zygomorphic development found in this investigation may be due to mechanical contact of the smaller embryo against the larger one. Only the smaller embryo of the two was affected, and in each case the shorter cotyledons were found to be situated on the side which was in contact with the larger embryo. Buchholz (1) draws some deductions regarding a zygomorphic development observed in the larger embryo which he considers as evidence that polycotyledony may have given rise to dicotyledony and monocotyledony. However, the present observations suggest the possibility that such factors as mechanical pressure during development may have an influence in bringing out such irregularities.

Pinus sabiniana

Observations of the results of 94 seeds of digger pine, which germinated, revealed two instances of two well developed seedlings from single seeds. One pair of the seedlings is shown in figure 3, as they appeared when discovered. The cotyledons of the one were erect and free from the endosperm while the cotyledons of the other were wrapped around the stem of the larger seedling.

The other pair of seedlings from a single seed of *Pinus sabiniana* developed in a control planting in which the seeds had not been chilled. One of these seedlings appeared six days before the other. Each seedling had 17 cotyledons. The smaller seedling showed a zygomorphic tendency in that it had eight short cotyledons; furthermore, it was only 4 cm. long with a poorly developed root, yet its young leaves appeared to be growing well. The larger seedling was 17 cm. long, bearing evenly developed cotyledons, and had a root twice as long as that of its "twin," but it had no visible leaves.

Pinus cembroides var. monophylla

Two instances of two well developed seedlings from single seeds were observed when examining the results of the germination of 111 seeds of one-leaf piñon. One pair of these seedlings is shown in figure 5, as it appeared above the soil with the cotyledons enclosed within the seed coat. Removal of the seed coat showed that the endosperm had been entirely digested.

A summary of the results of the observations showing the rarity of the occurrence of multiple seedlings in three species of the genus *Pinus* is given in table 1. Where two seedlings occurred from the same seed there must

TABLE 1. *A Summary of the Observations of Multiple Seedlings*

Species	Total Number of Germinated Seed	Total Number of Sets of "Twin" Seedlings in Each Species
P. torreyana	173	2
P. sabiniana	94	2
P. cembroides var. monophylla	111	2

have been two well developed embryos. The dissection of ripe seeds which follows shows two such embryos in each of two species.

RESULTS OF DISSECTION

Pinus torreyana

Fifty untreated seeds of *Pinus torreyana* were taken at random from the general stock and dissected to determine the nature of polyembryony and its relative frequency. Only one seed showed polyembryony. In it two embryos were found equal in their development. Each was 5 mm. in length. The cotyledons were equally developed on both. One embryo was directly above the other in such a position that its root tip was partly surrounded by the cotyledons of the other. The lower embryo was not in a healthy condi-

tion as it was somewhat brown in color; therefore, it probably would not have developed further had the seed been placed in favorable conditions for germination. The seeds of this species are oval, but more or less angled. Their average size is 10 by 20 mm.

Pinus sabiniana

The seeds of *Pinus sabiniana* were larger than those of *Pinus torreyana*, being 13 by 20 mm. The seed coats were about 2 mm. thick and were of a dark brown color, almost black, and mottled more or less with a light yellowish brown. Fifty seeds of *Pinus sabiniana* which were dissected showed one instance of polyembryony which is illustrated in figure 4, showing the nature and relative position of the embryos.

Pinus cembroides var. monophylla

The seeds of one-leaf piñon are oval, about 12 by 23 mm. in size and of a chocolate color which changes to a rich mahogany after they are soaked in water for a time. The seed coats were only one-eighth of a millimeter in thickness. The seeds of this species which were used for dissection were those which floated on the surface of the water when the selection of seeds was made for the germination tests.

The dissection test showed that the endosperms of eleven of the fifty seeds were dry and hard, while 39 were apparently in good condition. Seven of the latter group showed from two to six embryos in each seed. Plate XLVI, figure 6, shows two embryos of equal development. The suspensor of the upper embryo was either absorbed or crowded in between the cotyledons of the lower embryo. Figure 7 shows six embryos from one seed, two of which failed to differentiate cotyledons. The largest embryo was 14 mm. in length possessing 9 cotyledons; while the smaller embryo at the side was 7 mm. long and showed 3 unequally developed cotyledons, the one nearest the larger embryo being the smallest. The smallest embryos with cotyledons were from 1 to $1\frac{1}{2}$ mm. in length. Their cotyledons were small but evenly developed. In each of three other polyembryonic seeds, two embryos attained complete differentiation. Figure 8 shows two such embryos with a linear arrangement. Here, a third smaller embryo occurs to the left below. Figure 9 shows only two embryos. The larger one is 13 mm. in length while the smaller one is 2 mm. long.

It is probable that these seeds of one-leaf piñon which floated on the surface of water were less mature than those which sank to the bottom. The immature seeds would have more space left within the testas if there was air enough present to float them. The lack of crowding within the testas might possibly constitute evidence that embryonic selection was not so intensive, permitting the survival of some of the embryos ordinarily aborted.

Pinus Coulteri and Pinus tuberculata

Fifty seeds of each of the above species, Coulter pine and knob-cone pine, were dissected but no evidence of polyembryony was found. Due to the small number of seeds dissected these negative results are not to be interpreted to mean that polyembryony does not occur in these species.

The summary of the results of the seed dissection in table 2 suggests that where two embryos of equal development occur within the same seed, such "twin" embryos have survived the usual elimination which Buchholz (1) found in small seeded conifers. Furthermore, such "twin" embryos might be expected to give rise to multiple seedlings. A study of the endosperm might yield information which would explain to some extent why there is this apparent difference between the small seeded and large seeded pines.

TABLE 2. *Results of the Dissection of 50 Seeds of Each of Three Species of the Genus Pinus*

Species	Shape and Approx. Size of the Seeds	Polyembryony
P. torreyana.....	Oval; more or less angled; 10 × 20 mm. seed coats 1 mm. in thickness.	2 embryos in one seed, each 5 mm. in length, linear arrangement.
P. sabiniana.....	Bluntly wedge shaped; 13 × 20 mm.; seed coats 2 mm. in thickness.	2 embryos in one seed; unequal in development. Fig. 4.
P. cembroides var. *monophylla*.....	Oval; 12 × 23 mm.; seed coats 0.125 mm. in thickness.	2 to 6 embryos were found in seven seeds; one seed contained 2 embryos of nearly equal development.

The extent of the elimination of the endosperm may be important. It seems probable that if the embryos remain relatively small at maturity accompanied by slight elimination of the endosperm, such a condition would be favorable to the survival of two embryos rather than one.

Degree of Elimination of Endosperm

Seeds of *Pinus torreyana* and *Pinus cembroides* var. *monophylla*, after one year of storage at room temperature, were selected for this determination to represent the large seeded species, while *Pinus banksiana* represented the small seeded group. *Pinus torreyana* and *Pinus cembroides* var. *monophylla* had been for one year at room temperature, but *Pinus banksiana* seeds had just been received from the Chippewa National Forest, Minnesota, and were collected in November 1929. The seeds had been tested at the Forestry Station and gave 59 percent germination in 33 days.

Embryos with endosperms were weighed intact and then each was weighed separately and the results for all three species are given in table 3, where a comparison of the elimination quotients is also made.

TABLE 3. *Average Degree of Elimination of the Endosperm from 60 Seeds of Each of Three Species of the Genus Pinus*

Species	Total Weight of Parts from 60 Seeds Expressed in grams			Ratio
	A Endosperms with Embryos Intact	*B* Endosperms Only	*C* Embryos Only	$\frac{C}{B}$
P. torreyana	16.011	13.695	2.316	0.1690
P. cembroides var. *monophylla*	20.000	19.810	0.190	0.0095
P. banksiana	0.1957	0.1700	0.0240	0.1410

The quotients obtained by dividing the weight of the embryos (C) by the weight of the endosperms (B) show that there is greater elimination of the endosperms of *P. torreyana* and *P. banksiana* than of *P. cembroides* var. *monophylla*. Furthermore, the data show that the embryos of the latter are much smaller than those of the former. It is probable that the above embryo-endosperm ratios indicate a more favorable condition for the development of multiple embryos in *Pinus cembroides* var. *monophylla* than in either of the other species. The quotient (Q) apparently varies according to the size of the embryo and the amount of endosperm, regardless of the size of the seed.

$$Q \text{ varies as } \frac{C >}{B <} \quad \text{or} \quad \frac{C <}{B >}$$

and polyembryony (P) apparently increases as the embryo remains small and the endosperm continues less diminished.

$$P > \text{as} \frac{C <}{B >}.$$

This may not prove to be true for the genus as a whole and it will be necessary to put as many seeds of the genus to the test as possible to determine the point.

DISCUSSION

The occurrence of more than one seedling from the germination of a single Gymnosperm seed is considered rare (6, 9). However, two seedlings from the same seed were found in each of the three following species: *P. torreyana*, *P. sabiniana*, and *P. cembroides* var. *monophylla*. The occurrence of more than one well developed embryo in a mature seed is also rare. Mottier (8) found five embryos in a mature seed of *Pinus edulis* but only two had cotyledons. Buchholz (1) states that, although polyembryony is such an extensive phenomenon in *Pinus*, he has never found a mature seed with 2 *fully* and *equally developed* embryos; one was always considerably larger than the other, but these were not common in the smaller seeded species. The seven species which he studied were *Pinus banksiana*, *P. laricio*, *P. echinata*, *P. Strobus*, *P. sylvestris*, *P. ponderosa*, and *P. edulis*. The

last two species were studied only in the very early stages, long before cotyledons begin to form. In each of the somewhat larger-seeded species, *Pinus torreyana* and *P. cembroides* var. *monophylla*, of this investigation, two instances of two well developed embryos in the same seed were found. In the former species the two embryos were of equal development; while in the latter species they were nearly equal in their development. Two embryos of equal size have been found in *Ginkgo* seeds by Lyon (7), but it is very doubtful if they have been observed in the seeds of *Pinus* previous to the observations reported here. It appears, therefore, that the usual elimination resulting from embryonic selection in the large seeded species of *Pinus* is not so intense as in the small seeded species where only one embryo survives. This explains to some extent the development of the two seedlings from the same seed.

However, as to their origin there are two possibilities, just as is the case among human twins (Newman, 9). If they are derived from two different eggs, the pine embryos may be considered "fraternal" twins or multiples; when derived from the cleavage of a single egg they constitute "identical" twins. Unfortunately we are at present unable to distinguish the identical twins in these pines from the fraternal twins at the time of their maturity as seedlings or embryos in a seed.

Concerning their origin Buchholz (1) has given an account of the development of the early embryo stages. As a rule each of the fertilized eggs of all pines thus far investigated (4) gives rise to a system of eight cells which he has designated as embryo initial cells. Four of these are called the rosette cells giving rise only to abortive embryos, since these are so situated that they are very unlikely to survive beyond the earliest stages. The other four initial cells give rise to primary embryos any one of which may ultimately survive to maturity. However, since more than one egg may be fertilized, there are usually several embryo systems combined into the polyembryonic complex which may be observed advantageously only in the immature stage. By the time they reach greater maturity as represented in the seed their origin as identical or fraternal twins can no longer be established, since the suspensors by which their origins are traced are partially crushed. The many embryos compete with each other for supremacy in a process of embryonic selection (3, 4) which results in the elimination of all or nearly all of the embryos by the time the seed development is completed.

In the seeds of *Pinus* which we have investigated in the present studies this elimination is less complete than in the species investigated by others. The question of whether the "twins" observed in germinated seeds were identical twins, belonging to the same embryo system, or fraternal twins, derived from different embryo systems must therefore remain undetermined.

The degree of the elimination of the endosperm was determined for three species, *Pinus torreyana*, *Pinus cembroides* var. *monophylla*, and *Pinus banksiana*. *Pinus torreyana* of the large-seeded pines and *P. banksiana*, a small-

seeded species, have relatively larger embryos with smaller endosperms than found in *P. cembroides*. However, Buchholz (1) reported polyembryony as being rather common in *P. banksiana* in the immature seeds but no indication of it was observed in the sixty mature seeds dissected for this test. Germination of *P. torreyana* seeds has twice resulted with two seedlings developing from one seed, while two embryos of approximately equal development were found in one seed. In comparison with this the dissection results of *Pinus cembroides* var. *monophylla* show 7 out of 39 good seed had from 2 to 6 embryos with cotyledons in each seed, and two seedlings developed from one seed in two instances. The endosperm elimination quotient for this species is about 1/15 or 1/17 of the quotient of the other two species. This small elimination quotient obtained for the seeds of *Pinus cembroides* var. *monophylla* may indicate a condition that favors the development of multiple embryos. The greater number of such embryos in this species also favor this view. There are probably at least two factors to be taken into consideration with respect to the occurrence of polyembryony in addition to those found by Buchholz, and these are (1) the size of the entire seed and (2) the quotient or comparative size of the embryo and endosperm.

Summary

1. Six instances of the occurrence of multiple seedlings are recorded; two were found in each of the three species: *Pinus torreyana*, *Pinus sabiniana*, and *Pinus cembroides* var. *monophylla*.

2. The occurrence of two embryos of equal or nearly equal development was found in each of two dissected seeds, one pair of equal development occurred in *Pinus cembroides* var. *monophylla*, while the other pair of nearly equal development occurred in *P. torreyana*.

3. A linear arrangement of the embryos was found in those seeds where two embryos of equal or nearly equal development occurred, one embryo being directly above the other.

4. Six embryos were found in one seed of *Pinus cembroides* var. *monophylla;* four of these embryos had cotyledons.

5. Polyembryony in each species usually showed selective development, one embryo being much larger than the others.

6. Where two seedlings or two embryos of equal development occurred from or in the same seed selective survival was less intense than in the small seeded species.

7. Evidence of a zygomorphic development appeared in some of the smaller embryos of *Pinus torreyana* and *P. sabiniana* which may have been due to contact and mechanical pressure against the sides of the larger embryo.

8. Fifty seeds of *Pinus Coulteri* and *Pinus tuberculata* were dissected but no evidence of polyembryony was observed.

9. The small quotient of the embryo-endosperm ratio for the seeds of

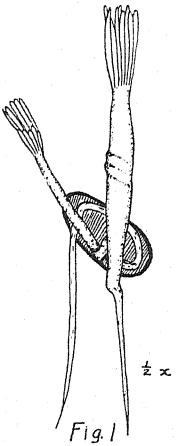


Fig. 1

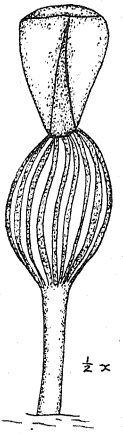


Fig. 2

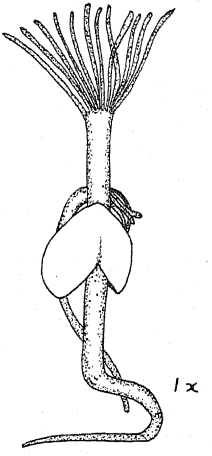


Fig. 3

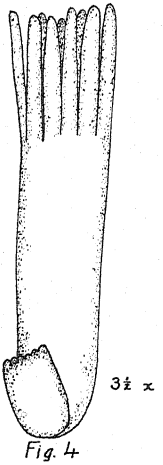


Fig. 4

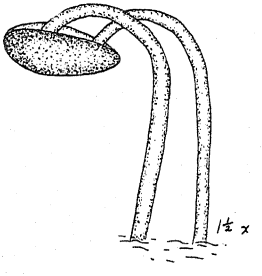


Fig. 5

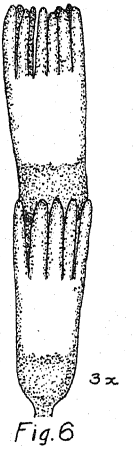


Fig. 6

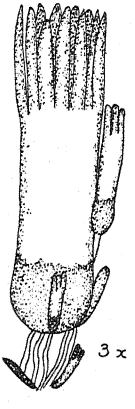


Fig. 7



Fig. 8

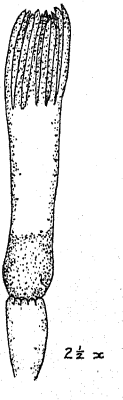


Fig. 9

Pinus cembroides suggests a condition which might favor the development of multiple embryos, but the data presented are insufficient for a definite conclusion.

10. More instances of the occurrence of multiple embryos occurred in the seeds of *Pinus cembroides* var. *monophylla* than in any one of the other species studied.

Acknowledgment is accorded Dr. John T. Buchholz for reviewing the manuscript and for suggestions.

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EXPLANATION OF PLATE XLVI

FIG. 1. Twin seedlings of *Pinus torreyana* with one-half of the seed coat and a portion of the endosperm attached.

FIG. 2. The normal development of *Pinus torreyana* with the endosperm and seed coat attached to the cotyledons.

FIG. 3. Twin seedlings of *Pinus sabiniana* with a portion of the endosperm attached.

FIG. 4. Twin embryos from a seed of *Pinus sabiniana*.

FIG. 5. Twin seedlings of *Pinus cembroides* var. *monophylla* with the cotyledons enclosed by the seed coat.

FIG. 6. Twin embryos of equal development dissected from a seed of *Pinus cembroides* var. *monophylla*.

FIG. 7. A group of embryos, four possessing cotyledons, dissected from a single seed of *Pinus cembroides* var. *monophylla*.

FIG. 8. The linear arrangement of embryos of unequal development, but from a single seed of *Pinus cembroides* var. *monophylla*.

FIG. 9. The linear arrangement of the embryos showing selective development which were dissected from a single seed of *Pinus cembroides* var. *monophylla*.

CRASSIPES, A NEW GRASS GENUS FROM UTAH

JASON R. SWALLEN

(Received for publication March 30, 1931)

There was recently received at the U. S. National Herbarium a peculiar grass from Utah which is generically distinct from any previously known. The name given to this new genus refers to the thick rachillas and short thick pedicels of the spikelets.



TEXT FIGS. 1–4. *Crassipes annuus*. FIG. 1, plant, natural size; FIG. 2, raceme showing rachis, × 2; FIG. 3, spikelet × 10; FIG. 4, lower floret and part of upper floret, glumes removed, showing rachilla joint and part of palea, × 10.

Crassipes Swallen, gen. nov.

Spiculae 3-florae, in racemo simplici unilaterali, flore superiore imperfecto; rachilla continua, lata, translucida; glumae latae, obtusae, marginibus hyalinis, prima 3-nervis, tertiam partem spiculae aequans, secunda 7-nervis, dimidiam partem spiculae aequans; lemmata obtusa marginibus hyalinis, nervis 5 prominentibus; palea truncata hyalina quam lemma paulo brevior, carinis scabris. Stamina 3.

Plantae annuae, racemis brevibus compactis.

Spikelets 3-flowered, the upper one imperfect, the rachilla continuous, broad, thickish, translucent; glumes broad, obtuse, with hyaline margins, the first 3-nerved, one-third the length of the spikelet, the second 7-nerved, half the length of the spikelet; lemmas obtuse with hyaline margins, 5-nerved, the nerves prominent, nearly parallel; palea truncate hyaline, sharply keeled, the keels sparsely aculeate-scabrous. Stamens 3.

Low annual grasses with short, dense, spikelike racemes, the spikelets in two rows on one side of the broad, thickened, apparently persistent rachis, the pedicels very short and thick.

Type species, *C. annuus*.

Crassipes annuus Swallen, sp. nov.

Culmi erecti, 2–4 cm. alti; vaginae carinatae, glabrae; laminae 7–18 mm. longae, 1–3 mm. latae, conduplicatae, naviculares, marginibus scabris; ligula brevissima, truncata, vel obtusa, ad 2 mm. longa; racemi 1–2 cm. longi; spiculae 6–7 mm. longae; gluma prima 2.5–3 mm. longa, gluma secunda 3.5–4 mm. longa; lemma inferius 5 mm. longum; palea 4 mm. longa.

Plants in small tufts, the culms erect or somewhat spreading, 2–4 cm. tall; sheaths keeled, glabrous, the two upper ones 2.5–3 mm. wide from the keel to the hyaline margins; blades 7–18 mm. long, 1–3 mm. wide, conduplicate, the tips navicular, the keel and margins sparsely scabrous, otherwise glabrous, the junction with the sheath obscure, the upper two exceeding the racemes; ligule truncate and very short or obtuse and as much as 2 mm. long; racemes partly inclosed in the upper sheaths, 1–2 cm. long; spikelets compressed, 6–7 mm. long, the pedicels 0.5 mm. long; first glume 2.5–3 mm. long, the second 3.5–4 mm. long; lower lemma 5 mm. long; stamens yellow, 1–1.2 mm. long.

Type in the U. S. National Herbarium, no. 1469998, collected "by the side of the open drive" between Salt Lake City and Ogden, at the foot of the Wasatch Mountains, April 26, 1928, by C. W. Fallas.

Dry sandy or gravelly soil, Utah and Idaho.

UTAH: Between Salt Lake City and Ogden, C. W. Fallas.

IDAHO: Moscow, F. W. Gail.

Crassipes belongs to the tribe Festuceae, subtribe Seslerieae. It is most closely related to *Oreochloa*, a European genus, but differs in having thick axes, rachillas, and pedicels, fewer-flowered spikelets, and firmer, more strongly nerved lemmas. It is near the North American genus *Orcuttia*, but is at once distinguished by the entire glumes and lemmas.

BUREAU OF PLANT INDUSTRY,
UNITED STATES DEPARTMENT OF AGRICULTURE

POLLEN-TUBE BEHAVIOR IN *BRASSICA PEKINENSIS* WITH REFERENCE TO SELF-INCOMPATIBILITY IN FERTILIZATION

A. B. STOUT

(Received for publication April 1, 1931)

In the flowers of *Brassica pekinensis* the styles are short and the stigmas are close to the ovary. The styles are solid and the pollen tubes travel through a conducting tissue (figs. 5 and 6, Pl. XLVII). Self-incompatibility in fertilization is strongly developed in *Brassica pekinensis*. In the first plants studied by the writer many plants set no seed to self-pollination; some, however, set seed rather abundantly to selfing. Repeated selection for high grades of self-seeding was continued for seven generations involving a total of over 5000 individuals and in all cases some plants completely self-incompatible appeared in the progeny (Stout, 1922; 1927).

No individual plant was found which was fully self-fertile throughout its entire period of bloom. There was for all such plants, grown under conditions of vigorous growth, a cyclic and synchronous development of mid-period self-fertility, and at the beginning and at the end of the flowering period usually no seeds were produced to self-pollination. There were many grades of fruit and seed production in regard to the number and size of the capsules and whether or not viable seeds were produced. Except for flowers that were aborted or proliferated it was shown that large capsules with viable seeds could be obtained from compatible cross-fertilization. It is the pollen-tube behavior in this cyclic change from self-compatibility to self-incompatibility that is especially reported in this paper.

METHODS

Typical plants of pedigreed cultures of *Brassica pekinensis* reared in pots in a greenhouse were selected when the first flowers began to open and the inflorescence of each was enclosed within large "glassine" bags, which are almost transparent, in order to prevent chance cross-pollination. Each day between 10 and 11 A.M. the freshly opened flowers on the main axis, usually from two to six in number, were self-pollinated by hand. After considerable preliminary study it was learned that the pollen tubes make little or no further growth after a period of 24 hours, and hence for the investigations here reported all flowers studied were removed from the plant 24 hours after they had been pollinated. About half of the flowers, every other one as a rule, were removed and preserved for study, the others being left to reveal by the subsequent development of the ovary the grade of compatibility or incompatibility operating in each of them.

The paraffin method of study was utilized, but for most of the studies a method of direct dissection and staining was developed that is simple, rapid, and especially reliable for tracing the continuous course of the pollen tubes. As soon as the pistils were removed they were placed in a killing solution of 100 cc. of 70% alcohol and either 6 or 8 cc. of commercial formalin (37%). With such preservation the pistils remained in excellent condition for study for at least a year, but in most cases the material was studied as soon after preservation as was convenient.

The dissections of pistils were made as follows: a pistil was placed on a glass slide and slit with a razor blade lengthwise into halves which were then arranged with the cut surfaces uppermost. Sometimes a half was also sectioned. Another method used was to slit the pistil its entire length with a fine sewing needle mounted in a wooden handle. The sections were made either at right angles or parallel to the central placenta as desired.

Numerous stains were tested in various combinations and with various methods of application before a satisfactory differential stain for the pollen tubes in the pistils was obtained. The method found to be most fully satisfactory for *Brassica* is as follows. Aceto-carmine solution made by saturating warm 45% acetic acid with carmine is dropped on the dissected pistils and allowed to remain for a few seconds, after which a few drops of a saturated aqueous solution of aniline blue are added. After a few seconds, depending on the density of stain desired, the excess stain is drawn off either with a pipette or with blotting paper. A few drops of aqueous solution of magenta red applied before the aniline blue sometimes improves the differentiation of the pollen tubes. Any desired degree in the density of the stain is then obtained by washing with absolute alcohol applied from a pipette. A drop of glycerine and a cover glass complete the preparation.

With the use of a microscope the extent of the growth of the pollen tubes in the various pistils was determined. If any tubes were found in the extreme lower part of the style the ovary was also sectioned and stained. By this method it was rather easy to determine the position of the ends of the pollen tubes in the pistil and their appearance.

Pollen-tube Behavior

The Structure of the Pistils and the General Behavior of Pollen Tubes

The pistils in flowers of *Brassica pekinensis* usually range from 5 to 7 mm. in length with the ovary approximately three-fifths of the entire length. The stigmatic surface is broadly rounded with a shallow funnel-like depression in the center and the entire surface is covered with papillate cells. There is no obvious accumulation of secretion; the secretion evidently exists as a thin film. At the time when the flower is only partly open the papillate cells of the stigma are slender and closely pressed together, but as the flower becomes fully open the stigma becomes broader and the protruding parts of the papillae are spread apart somewhat (Pl. XLVII, fig. 5). The

style is cellular throughout. From the base of the papillae a funnel-shaped group of cells leads to a central cylinder of closely compacted elongated cells which extends through the style (figs. 5 and 6). In compatible fertilizations the pollen tubes travel in this cylinder, pass into the ovary chamber, travel over the walls of the placenta, and thus reach the micropyles of the various ovaries within a period of 24 hours.

The short styles in the flowers of *Brassica pekinensis* are not favorable material for studies of the hourly rate of growth of the pollen-tubes or of the distribution of the tubes in the pistil. Also throughout much of the period of flowering for plants with cyclic changes in compatibility the successive flowers are in different conditions as to the grade or degree of self-compatibility. Therefore the studies made with these plants aim only to determine the extent to which the processes of fertilization proceed in the successive flowers, and as this usually appears fully to be realized within a period of 24 hours the pistils were removed for study at the end of this time-interval. By studying every second or third flower of the main axis, data are obtained on the various types of pollen-tube behavior throughout the cyclic changes of self-incompatibility and self-compatibility.

Pollen-tube Behavior During Self-incompatibility

The reactions of self-incompatibility in flowers of *Brassica pekinensis* involve (1) a low percentage of germination of pollen on the stigma, (2) coiling of pollen tubes on the papillae, (3) feeble or limited growth of tubes through the style, (4) coiling of the ends of tubes in the pistils, ovary, or ovules, or (5) combinations of various of the reactions just named.

The conspicuous coiling of the pollen tubes is a most characteristic reaction. In extreme cases of this (figs. 1 and 2) a tube will make several closely compressed coils which sit upon the stigma and seem appressed to a papilla and adhering to it. In many cases the treatment of preparations removes the grains with their coiled tubes; but frequently they remain in place even after all the processes of the paraffin method (fig. 5). In most cases the coiled tubes remain in this condition and make no further development. When a plant is at all times self-incompatible, a large percentage of pollen tubes coil on the stigmas during the entire period of blooming. In cases of very feeble self-compatibility in which only small empty capsules are formed in the mid-period, a few pollen tubes may be found in the ovary cavity 24 hours after pollination. It is clear that fertilization and seed-production do not necessarily follow when tubes penetrate into the ovary.

It should be noted that in their studies of pollen-tube behavior in *Brassica oleracea*, Sasaoka (1928) and Kakizaki (1930) report no coiling; the former states that pollen tubes grow to the ovary in 26 hours even when there is self-pollination, but it is not indicated that he studied any individuals fully self-incompatible. The latter states that, in general, the incompatibilities in the plants he studied involve a slow rate of growth of pollen tubes.

In the mid-period of blooming when the flowers of certain plants set seeds to self-pollination relatively few or even no pollen tubes become coiled on the stigma and at least some of the tubes pass directly through the style (fig. 3) into the ovary and enter the ovules through the micropyle all within 24 hours after pollination. It is the rule, however, that large numbers of pollen tubes do not enter the ovary chamber. The number of seeds in the largest of capsules to either self- or cross-pollination is seldom as many as 30, hence a full capsule of seeds does not necessarily involve penetration into the ovary for the majority of the pollen tubes that start growth on the stigma.

In those plants that show extreme cyclic change of incompatibility the production of capsules and seeds to controlled self-pollinations gives a remarkably well-defined gradation from complete self-incompatibility to the highest degree of self-fruitfulness (see Stout, 1922) in somewhat the following order: (1) the ovaries do not enlarge and the pistils soon fall; (2) the pistils remain attached for some time and may enlarge somewhat; (3) there is a noticeable enlargement of the ovary but no seeds are formed; (4) the capsules become larger and seeds start to develop but abort; (5) viable seeds are produced in varying numbers; and (6) seed production reaches a climax giving large capsules well-filled with viable seeds. Following this there is a series of stages in the reverse order to the above. In addition to the cycle just noted some of the first flowers that appear may be blasted.

The study of pistils collected throughout the entire period of blooming reveals various combinations of pollen-tube behavior which correlate with the development of ovaries and ovules. During the feeble development of ovaries no pollen may germinate or there may be germination but some tubes may coil on the stigma while others proceed without coiling and extend into the style for short distances. The relative proportion of pollen tubes that coil and of those that do not coil varies greatly. When plants have a short mid-period of self-compatibility of a low degree there is usually considerable coiling of pollen tubes on every pistil. In general the number of seeds produced in capsules is closely and directly related to the proportion of coiling observed.

Coiling of pollen-tubes is not confined to the stigmatic surface. Rather frequent instances of decided coiling at the ends of pollen tubes have also been observed in the stylar tissues, on the walls of the placenta, in the region of the micropyle, and within the ovule in the immediate vicinity of the embryo sac or even in contact with it (fig. 4). Often the pollen tubes follow a very indirect course over the walls of the placenta.

Results when Stigmas are Treated with Water

Yasuda (1928) reports that in *Petunia violacea* removal of the secretions on pistils by means of water may change the reactions in a pistil from self-incompatibility to self-fertilization. However, it appears that the pollen tubes from self-pollination of self-incompatible plants penetrate to a distance

of about one-fifth of the style, so the influence of the stigmatic fluid does not entirely inhibit growth of the tubes as in the case of extreme self-incompatibility in *Brassica pekinensis*.

A secretion does not collect to form a droplet or a conspicuous film on the stigmas of *Brassica pekinensis*. The stigmas of a series of flowers were, however, treated with several applications of water and the film of water remaining on the stigma was removed with blotting paper before pollination. In no case was there a change in the compatibility or incompatibility as judged by the behavior of untreated flowers open on the same plant on the same day. Flowers thus treated set fruit with seeds to self-pollination provided the flowers opened during the period of mid-period self-compatibility, and hence the treatment did not interfere with normal pollen-tube growth; but during the periods of self-incompatibility no seeds were obtained to the self-pollination of pistils thus treated.

The Germination of Pollen on Artificial Media

The best artificial germination of pollen of *Brassica pekinensis* thus far obtained has been on a medium made of 100 cc. water, 2 grams agar agar, and 15 grams cane sugar. The tubes grew to an average length of about 250 micra and there was no coiling in any pollen tube at any time.

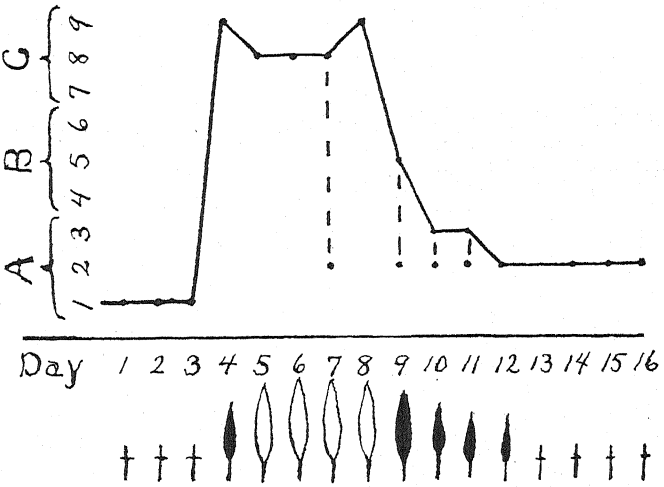
Summary and Discussion

The extreme expression of self-incompatibility in *Brassica pekinensis* involves powerful inhibition of pollen germination and pollen-tube growth on the stigma. There is the failure of self-pollen to germinate and there is a conspicuous coiling of short tubes in definite reactions on the surface of the stigmatic papillae. For a large number of plants the inhibition of the normal growth of tubes from self-pollen is complete for all flowers throughout the entire period of blooming. Here the factors for incompatibility completely inhibit fertilization in selfing.

For another group of plants there is the development of capsules and seeds to self-pollination during the mid-period of bloom. Previous to and following this period there is a decrease in the efficiency of self-pollination and at least some of the first flowers and some of the last flowers to open normally are, as a rule, either fully self-incompatible or nearly so. In the flowers of these plants pollen-tube behavior is closely correlated with the results observed in seed production. During the mid-period of self-compatibility the pollen tubes grow to the ovary within 24 hours and fertilization is completed. In the end-periods of complete self-incompatibility there is either failure of pollen to germinate or there is a pronounced coiling of the tubes. In the transition periods some pollen tubes may coil and some may proceed into the pistil, and coiling reactions of the tip of pollen tubes are to be seen in the style and even within the micropyle. But seeds do not necessarily form when pollen tubes are within the ovary and hence some degrees

of incompatibility or of infertility operate in the later stages of fertilization. That there is an inherent and genetic basis for the two types of behavior to self-pollination may be assumed: but the plants do not fall into two distinct groups; there are many gradations in the degree of self-compatibility. Also in no case of the large number of plants studied has any plant been completely self-compatible in that all its flowers set seed to self-pollination.

It has already been reported (Stout, 1922 and 1927) that selection for highest grades of mid-season self-compatibility in *Brassica pekinensis* was



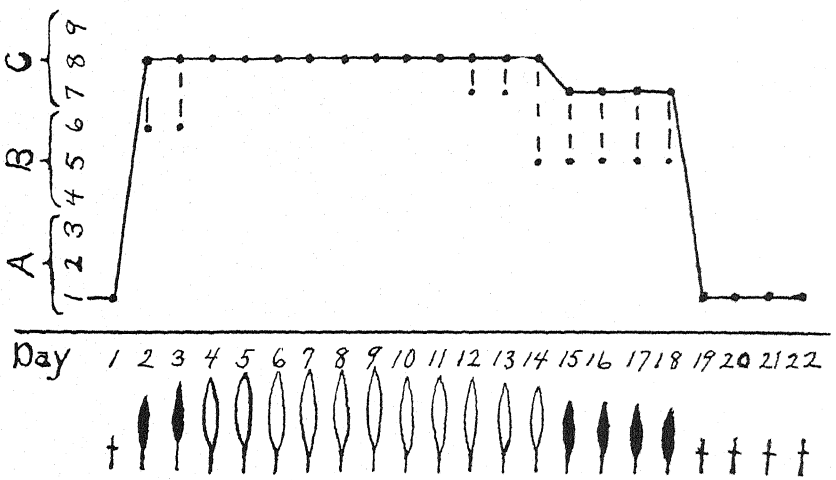
TEXT FIG. 1. Pollen-tube behavior and development of capsules for a plant somewhat self-compatible which had a blooming period of 16 days. The representation of pollen-tube behavior is as follows: *A*. Pollen on stigma: 1, no germination; 2, tubes coiling; 3, tubes penetrating. *B*. Pollen tubes in style: 4, entering style; 5, coiling; 6, no coiling. *C*. Pollen tubes in ovary: 7, coiling; 8, no coiling; 9, found in micropyles. For the development of capsules the barred line or cross represents no enlargement; the black capsule-like symbols represent capsules with no seeds; and the outlines of capsules represent capsules that gave viable seeds, and the relative sizes of these are somewhat indicated.

As shown in the diagram the plant in question was highly self-fruitful from the 5th to the 8th days of the flowering period during which pollen tubes were found in the ovary, but on the 7th day some tubes were also found coiling on the stigma. During the first three days there was no germination of pollen, and during the last five days there was much coiling of pollen tubes on the stigmas. For the flowers of the 4th day, pollen tubes entered the ovary and were found in the micropyle and none were found coiling on the stigma, but seeds were not formed in the ovaries of the flowers of that day. From the 8th to the 13th day there was a rather gradual transition in pollen-tube behavior which was closely correlated with the development made by capsules.

continued for seven generations during which a total of more than 5000 plants were grown and tested. With selection there was an increase in the number of plants that were highly self-compatible, but in no line did all plants become self-fertile and the end-periods of self-incompatibility were still present. Yet at least half of the plants were highly self-fertile and it

was evident that there was some genetic basis for this condition. It was found that cross-incompatibilities were also present in the groups of plants studied and there was some indication that the cross-relations may be cyclic.

It seems clear that the incompatibilities in *Brassica pekinensis* do not conform to either of the three main types recently recognized by Correns (1928). (1) In the *Tolmia* type exemplified by *Tolmia Menziesii* all individuals are self-incompatible but are fully cross-compatible, and hence the conditions which inhibit self-fertilization have no influence on cross-fertilization. (2) In the Cruciferous type exemplified by the results Correns (1913) obtained with *Cardamine pratensis* there was self-incompatibility and also



TEXT FIG. 2. Diagram for a plant highly self-compatible in a long mid-period of blooming. This shows graphically the behavior of a plant highly self-compatible from the 4th to the 14th day of bloom, throughout which pollen tubes were found in the ovaries with no coiling whatever on the stigmas. In relatively short end-periods there was complete self-incompatibility. During the transition periods covering the 2–3 and the 15–18 days of bloom, pollen tubes were in the style and in the ovary and there was more or less coiling, and only capsules with no seeds were produced. The rather close correlation between pollen-tube behavior and development of ovary is here shown, but it is to be noted that seeds are not necessarily produced when pollen tubes are within the ovary.

certain group reactions of cross-incompatibility in which pollen tubes react according to the diploid nature of the plant which produced them. Apparently there was no differential fertilization among sister pollen grains. (3) In the Personate type as seen in such genera as *Nicotiana*, *Veronica*, and *Verbascum* the genetic regulation of both self- and cross-incompatibilities and of cross-compatibilities and of fertilizations that occur are assumed to involve a control by oppositional factors (S_1, S_2, and S_3) according to the haploid genetic organization of pollen-tube nuclei in relations with the diploid pistils. This admits of a differential action among two classes of sister pollen in certain cross-relations. In all these three types it is assumed

that the members of the species are all genetically self-incompatible and that the many deviations from complete genetic determination of this condition are to be considered as phenotypic (see especially East and Park, 1917; and East, 1929).

Still more recently Kakizaki (1930) has recognized another type of genetic regulation of pollen-tube growth in incompatibilities and in normal fertilization in the results he obtained with head cabbage (*Brassica oleracea*). The analysis is in terms of two groups of special factors; one group (T_1 and T_2) favors and promotes pollen-tube growth, and one group (S_1, S_2, and S_3) of oppositional factors inhibits pollen-tube growth. Both qualitative and quantitative reactions are assumed for these factors in the reactions between diploid pollen-tubes and haploid pistils. This, it may be noted, provides an explanation for the behavior of a line of plants selected for high self-compatibility which continues to give self-compatible and self-incompatible plants in a ratio of approximately 1 : 1. Thus plants of the composition $S_2S_3T_1T_2$ which are feebly self-compatible can give offspring of $S_2S_3T_1T_1$ and $S_2S_3T_2T_2$ which are self-compatible, but the selfed offspring of each of these will be $S_2S_3T_aT_a$ which are self-compatible and $S_aS_aT_aT_a$ which are self-incompatible. Repeated selection of the $S_2S_3T_aT_a$ plants gives no race that breeds true to self-compatibility, which in general is the result obtained in *Brassica pekinensis*.

But the results obtained by Kakizaki show many grades of self-fertility in plants classed as self-incompatible, and many grades of incomplete fertility in plants classed as self-compatible. The relations of selfing and crossing were classed as incompatible if the "relative fertility" was less than 50 percent in a scale ranging from 0 to 180 percent. Of the eight parent plants of Succession cabbage, six produced some seeds to selfing. All eight of the parent plants of the variety Toyodawase judged as self-incompatible produced seeds to selfing and some rated over 40 percent. Of 125 plants of various progenies classed as self-incompatible, 94 were somewhat self-fruitful and 14 of them ranked higher than 30 percent. The same conditions existed for the cross-relations and about three-fourths of the cases classed as cross-incompatible produced at least some seeds. Such irregularities are frequent, if not the rule, for plants of species in which there are incompatibilities.

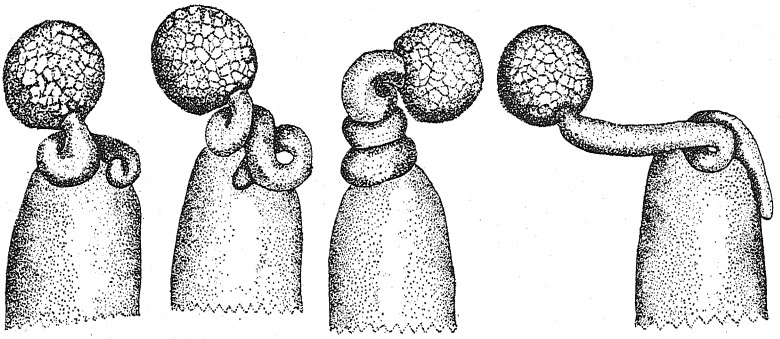
The very frequent occurrence of feeble and partial self-compatibility in relations that are assumed to be genetically incompatible is an anomalous condition in considering the genetical regulation of pollen-tube behavior. The significance of such results has been discussed by the writer (1916, 1920, 1922, 1927) and the conclusion made that in these cases the genetical factors for incompatibility are unstable. More recently East (1929) has suggested that there may be "borderline" genes which are variable in expression.

The variations that occur among the flowers of a single plant, such as end-season fertility, seed production in old flowers or from premature pollination,

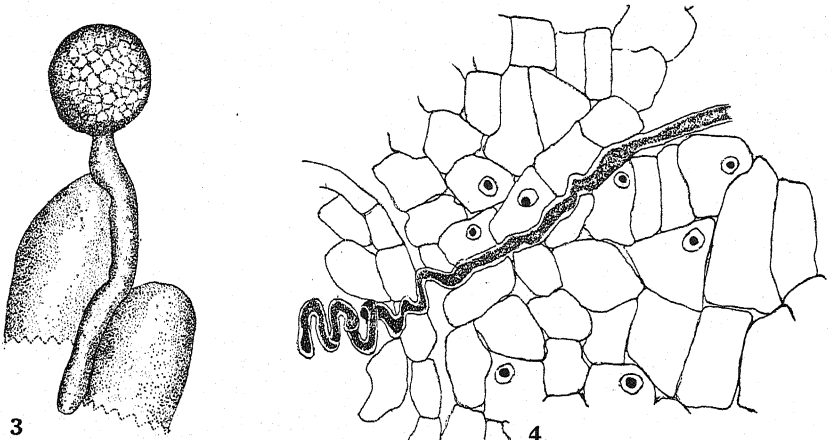
and cyclic mid-season fertility all suggest some phase of phenotypic development. To some of these phenomena the term "pseudo-fertility" has been applied (East and Park, 1917); but it is to be noted that the seed production here involves a normal fertilization, and since there are many grades of reduced fertility in relations judged as fully compatible there is on the same basis also a "pseudo-sterility." Kakizaki considers that, in addition to pseudo-fertility, the assumed allelomorphs "function in more or less different intensities, or that, besides these, one or more factors of minor value are involved."

It is not clear that there is in these cases merely quantitative phenotypic variation such as that of weight of seeds produced on a single plant of the bean. Instead, it seems that there is the interplay of at least two or possibly three rather distinct types of hereditary factors. It seems rather evident that there are present in the plant not only factors for incompatibility but also factors favoring fertilization. In any species, and especially in those that have homomorphic flowers, such as *Cardamine pratensis*, *Nicotiana alata*, *Brassica oleracea*, and *Brassica pekinensis*, there are those fundamental factors or characteristics in structural and in physiological adaptation for fertilization within species, and for which the species is generally homozygous. There may also be special factors favoring fertilization which operate to promote or to influence fertilization either of selfing or of selective crossing within the species (see especially "pollen-tube factors," Jones, 1928) and for these there may be heterozygosity. The factors for incompatibility are clearly a distinct group of factors which inhibit fertilization; they operate unfavorably to fertilization when there is similarity (as $FFS_1S_2 \times FFS_1S_2$) and they allow fertilization through the action of fundamental factors when there is dissimilarity (as $FFS_1S_2 \times FFS_2S_3$). With this conception, self-fertility and self-incompatibility are not to be considered as strictly contrasted characters in inheritance. The oppositional factors S_1, S_2, and S_3 are limited to inhibition reactions when there is for them similarity in genetic composition in pistils and pollen, as S_1S_2 pistils and S_1 and S_2 pollen. But when there is dissimilarity for them as S_1S_2 pistils and S_3 pollen there is inaction and the fundamental factors (FF) or the special sympathetic factors (T_1 and T_2) operate.

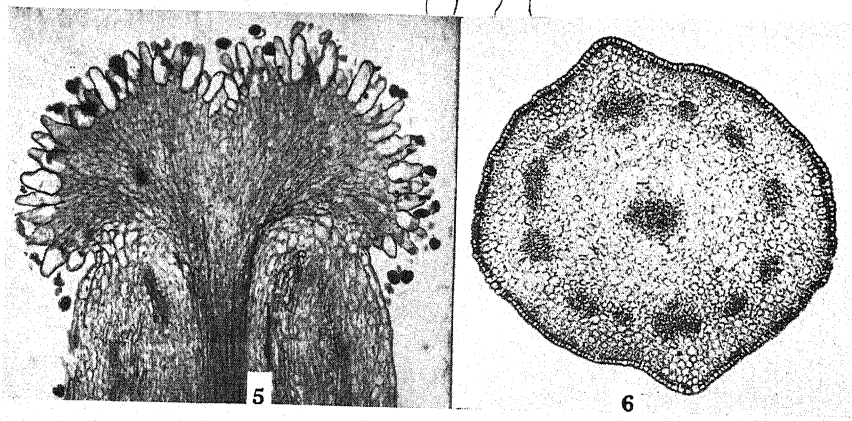
It is also evident that factors for incompatibility are often highly specialized and subject to limited or partial action both during the entire life of a flower and during the cycle of flowering of a plant. In *Petunia violacea* Yasada (1928) has shown that the reactions of self-incompatibility are so highly specialized and localized in the secretions of the pistil that the removal of the secretion leads to self-seeding. Many types of pseudo-fertility show limited and specialized action of the factors for incompatibility. In many individuals the combination of factors of two or more classes with quantitative and qualitative inter-reactions (for example $FFS_2S_3T_1T_2$) are such that some fertilizations may occur either in almost any flower or in such cycles as are seen in certain plants of *Brassica pekinensis*.



1 2

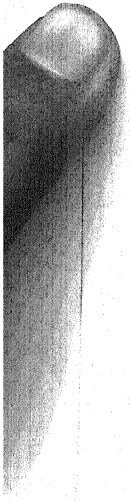


3 4



5 6

STOUT: SELF-INCOMPATIBILITY



The studies of pollen-tube behavior and of self-incompatibility here reported are a part of investigations which have been in progress for several years and various students and assistants have been associated with the work and have contributed much to it. The coiling of tubes on the stigma in *Brassica pekinensis* was first observed by Dr. Clarence E. Moore in connection with studies he was making on pollen-tube behavior. Further studies on *Brassica* were made by Dr. Clement G. Bowers and Mr. T. Susa. But all the data directly used in the graphs of this paper were obtained in 1928 and 1929 from a completed series of pollinations for each of the plants the technical preparations of which were made by Miss Clyde Chandler, technical assistant to the writer.

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EXPLANATION OF PLATE XLVII

All figures are of *Brassica pekinensis.*

FIGS. 1 and 2. Typical coiling of pollen tubes on stigmatic papillae during complete self-incompatibility. × 860.

FIG. 3. Pollen tube proceeding into style without coiling during mid-period of self-fertilization and self-seeding.

FIG. 4. Coiling of end of pollen-tube within embryo sac during transition to mid-period self-fertility. × 1300.

FIG. 5. Longitudinal section showing upper portion of style and the stigma with coiled pollen-tubes. × 80.

FIG. 6. Cross-section in middle part of style showing solid pistil and central cylinder of conducting tissue. × 80.

THE RELATION BETWEEN CATALASE ACTIVITY AND VIGOR IN INBRED STRAINS AND CROSSES OF CORN SEEDLINGS

H. L. CHANCE

(Received for publication April 1, 1931)

The wide distribution of catalase in the plant and animal kingdoms led Loew (1901) to assume that it had some definite function and to suggest that it might be associated with the respiratory processes. These processes have to do with energy release which in turn is associated with protoplasmic activity and possibly rate of growth. The rate of growth may be increased by the addition of nitrogen as may also the rate of catalase activity of apple leaves. The latter was demonstrated by Heinicke (1923) who found that the addition of sodium nitrate to a sod soil in which apple trees were growing was accompanied by an increase in the catalase activity of the leaves. The relative catalase activity was used as a basis for a viability test of various varieties of lettuce seed by Davis (1926). The test depended upon a retention or a reduction of the catalase of soaked seed as compared with that of dry seed of the same sample. Any considerable reduction in catalase was taken to indicate poorer quality of seed.

In the animal kingdom Burge and Burge (1921) found that catalase activity paralleled development in potato bugs and mice. Low oxidation in the newly born is attributed to the low catalase content while the intense metabolism characteristic of youth and adult life is attributed to high catalase content. In these determinations Burge and Burge macerated the whole eggs, larvae, pupae, and adults of potato bugs while the mice were skinned and all tissue except the skin used.

This association of catalase activity with viability of seed and an increased rate of growth is not always present, as some workers have pointed out, but it leads to the suggestion that there may be a parallel between the intensity of catalase activity and the vigor of seedlings.

In view of this suggestion inbred strains of corn were studied in the hope of being able to determine the vigor of a strain while in the seedling stage. The seed were from inbred strains and from crosses between them, the vigor of which, as measured by the total yield of dry matter expressed as a percentage of the total yield of the commercial stock and yield of grain, had been determined in field tests. In the present study only two partial measures of vigor were taken, each being considered independently. The first is the relative fresh weight of the tops of the seedlings and the second is the dry weight of the mature offspring of the inbred strains expressed as a percentage of the dry weight of the commercial stock from which the inbred

strain was derived. In the case of crosses, the second measure of vigor is expressed as a percentage of the dry weight of the better parent.

The work was started with a total of sixty-four strains and crosses. These were divided into eight groups. A single group was planted on each of the following dates: March 2nd, 6th, 12th, 31st, April 6th, 19th, 23rd, and 30th. Due to a delay in the standardization of method of procedure, the planting of March 2nd was discarded while only the data from the second harvest of the groups planted the 6th and the 12th of March were considered in the results. Data were obtained from five groups or forty plants for the first determination and from seven groups or fifty-six plants for the second determination. For convenience, the planting of the 6th of March is designated as Group I, of the 12th of March as Group II, etc.

Each group was grown simultaneously in sand and soil. Catalase determinations were made at two stages of development, when the green weights of the tops were roughly .4 and .9 grams, respectively. These were designated as the first and second determinations. In the first case all of the top was used and in the second the leaves and stems were treated separately. Thus comparisons can be made between the relative rates of catalase activity of the tops as a whole and between those of the leaves and stems of seedlings grown in either sand or soil.

An effort was made to keep the time of collection and preparation of material uniform with reference to the time of determination. In nearly all cases collections were made between twelve noon and two o'clock in the afternoon. The material was always prepared immediately after collection. In collecting, the stalks were cut off at the top of the coleoptile. Since it varied in length, there was some variation in the height at which the stalks were cut.

A slight modification of the standard method adopted by Heinicke (1923) was used. After preparation the suspension was placed in small bottles, tightly stoppered, and stored in a dark chamber at room temperature for approximately twenty-four hours before the determinations were made. The apparatus for the determination was adjusted so that the wheel operating the reaction tube made 240 revolutions per minute. The water bath was maintained at a temperature of 15° C. ± 1° C. Readings were taken in terms of seconds required to liberate certain volumes of gas. The time required to liberate a volume of one cubic centimeter was taken as a standard of comparison. The results are based upon the average of duplicate determinations.

In order to facilitate the study of catalase activity in relation to the measures of vigor chosen, the data for the seedlings of Group VI grown in sand are shown in table 1.

The seedlings are arranged in the order of their catalase activity. The last three columns of the table show the relative positions of the seedlings based first upon catalase activity, second upon the fresh weight, and third

TABLE 1. *The Relation of Catalase Activity in Seedlings Grown in Sand to Fresh Weight and to the Yield of the Mature Offspring Expressed as a Percentage of the Dry Weight of the Commercial Stock*

Plant No.	Fresh Weight, in Grams	Seconds for Evolution of 1 cc. of Gas	Relative Position Based on:		
			Catalase Activity	Weight	Percentage of Commercial Stock
26–120	.47	12	1	3	1
26–125a	.38	13	2	4	2
26–133	.16	20	3	8	3
26–127	.28	27	4	7	5
26–129	.51	55	5	1	4
26–122	.35	61	6	5	8
26–139	.32	69	7	6	7
26–135	.48	90	8	2	6

upon the yield expressed as a percentage of that of the commercial stock. The most active seedling was seven and one half times as active as the slowest although it weighed slightly less. The largest seedling weighed more than three times as much as the smallest, but the latter was nearly three times as active. A glance at the two columns shows lack of agreement throughout. Apparently there is better agreement between the catalase activity and the second measure of vigor since in four cases out of eight, the rank is the same.

The data for seedlings of the same strains grown in soil are shown in table 2.

TABLE 2. *The Relation of Catalase Activity in Seedlings Grown in Soil to Fresh Weight and to the Yield of the Mature Offspring Expressed as a Percentage of the Dry Weight of the Commercial Stock*

Plant No.	Fresh Weight, in Grams	Seconds for Evolution of 1 cc. of Gas	Relative Position Based on:		
			Catalase Activity	Weight	Percentage of Commercial Stock
26–120	.40	10	1	1 (2)	1
26–135	.18	12	2	8 (7)	6
26–125a	.21	22	3	5 (6)	2
26–133	.18	26	4	7 (8)	3
26–129	.34	35	5	3 (3)	4
26–127	.32	72	6	4 (4)	5
26–122	.40	86	7	2 (1)	8
26–139	.21	155	8	6 (5)	7

The seedlings ranking one and seven in catalase activity have the same fresh weight, but the first is more than eight and one half times as active as the latter. Those seedlings ranking two and four in activity have the same weight but the former is more than twice as active. Also the seedlings ranking three and eight in activity weigh the same although the first is more than seven times as active as the second. Since there are three duplicates in

weight, it makes possible two arrangements of the order or rank of the weights. The numbers not enclosed in parentheses give the order as used in the data. The seedling ranking highest in catalase activity also ranks highest on the basis of percentage yield of parent. While the relationship is not so marked as in table 1, there is some indication of a parallel between the catalase activity and the second measure of vigor.

Other seedlings of the same strains were allowed to grow six days longer and the catalase activity of the leaves and stems determined separately. The average activity of the leaves and stem of the same seedling was taken as the activity of the seedling as a whole. Here it may be mentioned that Knott (1927) has pointed out the inaccuracy resulting from the use of composite samples. The data for seedlings grown in sand are shown in table 3.

TABLE 3. *The Relation of Catalase Activity in Older Seedlings Grown in Sand to Fresh Weight of Leaves and Stem and to the Yield of the Mature Offspring*

Plant No.	Total Weight, in grams	Weight of:		Seconds for Evolution of 1 cc. of Gas			Relative Position Based on:		
		Leaf Tissue	Stem Tissue	Leaf Tissue	Stem Tissue	Average for Leaf and Stem	Catalase Activity	Total Wt.	Percentage of Commercial Stock
26–135	.94	.54	.40	17	26	21	1	5	6
26–122	.82	.50	.32	35	18	26	2	7	8
26–120	.85	.54	.31	39	29	34	3	6	1
26–133	.93*	.58*	.35*	59	39	49	4	8	3
26–139	1.08	.62	.46	81	43	62	5	3	7
26–127	1.10	.72	.38	95	107	101	6	2	5
26–125a	1.00	.58	.42	140	64	102	7	4	2
26–129	1.44	.96	.48	161	207	184	8	1	4

* Two small plants used.

The seedling ranking first in catalase activity ranks fifth in weight and sixth when the yield is considered as a percentage of that of the commercial stock. Similarly the least active seedling ranks one and four, respectively. Here there is no indication of agreement between the catalase activity and the first or second measure of vigor as was suggested in tables 1 and 2.

The activity of the leaf and stem tissue of the same seedling is not always of the same order though in many cases there is agreement. The average activity of all leaf tissue is slightly greater than that of all stem tissue.

The same type of data is shown for the leaves and stems of the same group of seedlings grown in soil in table 4.

The largest seedling ranks third in catalase activity and second on percentage yield basis. The one ranking first on the latter basis ranks second in catalase activity and second in weight. Since there are three duplicate weights of seedlings in table 2, it is difficult to make any comparison in the relative ranks of any seedling. In table 4 there is a greater divergence in

the order of the activity of the leaf and stem tissue than in the preceding table. Also the average activity of the leaf tissue is slightly higher than that of the stem tissue.

TABLE 4. *The Relation of Catalase Activity in Older Seedlings Grown in Soil to the Fresh Weight of Leaves and Stem and to the Yield of the Mature Offspring*

Plant No.	Total Weight, in grams	Weight of:		Seconds for Evolution of 1 cc. of Gas			Relative Position Based on:		
		Leaf Tissue	Stem Tissue	Leaf Tissue	Stem Tissue	Average for Leaf and Stem	Catalase Activity	Total Wt.	Percentage of Commercial Stock
26–135	.92	.45	.47	7	20	13	1	6	6
26–120	1.20	.72	.48	17	14	15	2	2	1
26–125a	1.30	.73	.57	26	16	21	3	1	2
26–133	.58*	.30*	.28*	35	19	27	4	8	3
26–122	.93	.56	.37	27	34	30	5	5	8
26–127	.74	.45	.29	23	39	31	6	7	5
26–129	1.20	.68	.52	25	46	35	7	3	4
26–139	1.14	.68	.46	53	35	44	8	4	7

* Two small plants used.

Correlation coefficients and probable errors were determined to show the relation of catalase activity to both measures of vigor. Coefficients were found for activity at both stages of development for the seedlings grown in both sand and soil. There were forty seedlings at the first stage and fifty-six at the second stage of development. The results are shown in the following table.

TABLE 5. *Correlation Coefficients Between the Relative Catalase Activity and Relative Vigor When the Fresh Weight is Taken as a Measure of Vigor (A) and When the Yield of the Mature Offspring Expressed as a Percentage of the Dry Weight of the Commercial Stock (B) is Taken as a Measure of Vigor.*

Grown in:	A Correlation Coefficient	B Correlation Coefficient
Sand, first determination....	-0.07 ± 0.10	0.20 ± 0.11
Soil, first determination.....	-0.07 ± 0.11	0.17 ± 0.11
Sand, second determination..	-0.14 ± 0.08	-0.33 ± 0.10
Soil, second determination...	-0.04 ± 0.09	-0.11 ± 0.11

The data show absence of significant correlations for all determinations for both measures of vigor. It will be noted that the sign of the coefficients is negative in most cases.

For further study the figures denoting the catalase activity of the seedlings were arranged in a column according to size and numbered serially. Two more columns were made and numbered in a similar fashion but using for the first the green weights of the seedlings, and for the second the figures denoting the yield of the mature offspring expressed as a percentage of the yield of the commercial stock. Using the serial number derived from the

relative catalase activity as a basis, the serial number derived from the fresh weight of the same seedling was placed opposite the catalase serial number. The correlation coefficient was then determined. In a similar manner the correlation coefficient between the catalase activity and the yield of the mature offspring expressed as a percentage of the yield of the commercial stock was determined. The results are shown in table 6.

TABLE 6. *Correlation Coefficients Between the Serial Number Derived from the Relative Catalase Activity, and (A) the Serial Number Derived from the Relative Fresh Weight and (B) the Serial Number Derived from the Figures Denoting the Yield of the Mature Offspring Expressed as a Percentage of the Dry Weight of the Commercial Stock*

Grown in:	A Correlation Coefficient	B Correlation Coefficient
Sand, first determination....	-0.11 ± 0.09	0.18 ± 0.11
Soil, first determination.....	-0.18 ± 0.13	0.22 ± 0.10
Sand, second determination..	-0.21 ± 0.09	-0.20 ± 0.11
Soil, second determination...	-0.15 ± 0.09	-0.19 ± 0.11

The results obtained are in agreement with those shown in table 5. The numbers indicating the correlation coefficients are too small in comparison with the probable errors to indicate significance.

The data in the tables show the relative catalase activity of eight strains constituting Group VI for four duplicate determinations. The position of each strain in each table is determined by its relative catalase activity. The most active strain is placed in the first position. It will be noted that the position of strain number 26–120 is fairly high in each table, ranking 1, 1, 3, and 2. Strain number 26–139 ranks fairly low in each table, ranking 7, 8, 5, and 8, respectively, while strain number 26–127 occupies an intermediate position, namely 4, 6, 6, and 6. The data seem to indicate that the position of a strain with reference to that of other strains under comparable conditions is fairly well maintained.

The total weight of forty seedlings grown in sand and soil was at the time of the first determination 15.87 and 16.67 grams, respectively, or an average of approximately .40 and .42 grams per seedling. The total number of seconds required for the liberation of one cubic centimeter of gas was 1564 for the seedlings grown in sand and 1669 for those grown in soil, the average being 39.1 and 41.7 seconds, respectively. From the standpoint of averages, the seedlings grown in sand were slightly smaller and more active than those grown in soil. The average difference in weight was .02 of a gram while the difference in activity was represented by 2.6 seconds.

A similar study of the leaf and stem tissues of forty-eight seedlings shows that the leaf tissue was slightly more active regardless of whether the seedlings were grown in sand or soil. The average activity of the leaf tissue of seedlings grown in sand was 44 seconds as compared with 47 seconds for the stem tissue. Figures for the leaf and stem tissues of seedlings grown in soil were 50 and 52 seconds, respectively. Striking an average, the time

becomes 47 seconds for leaf tissue and 49.5 seconds for stem tissue. If the average activity (48.25 seconds) of the leaf and stem tissue is taken as the activity of the whole seedling and this compared to the average activity (40.4 seconds) of the seedlings grown in sand and soil and harvested at the earlier stage of development, it will be seen that the younger seedlings gave on the average a greater relative activity. The data, as were those from the forty seedlings, were not treated statistically and the small difference may not be significant.

The data showing the relation of the catalase activity of inbred strains to that of the crosses of the corresponding strains were derived from the first determinations. Since the strains and crosses were not planted in the same flats and at the same time, the material is not strictly comparable. The data are not shown but no relation or parallel appears to exist between the catalase activity of the strains and that of their corresponding crosses. However, under comparable conditions, a parallel might be found.

Summary

1. When the fresh weight of inbred corn seedlings or the yield of the mature offspring expressed as a percentage of the yield of the commercial stock is taken as a measure of vigor, the data show no significant correlation between the catalase activity and the vigor of the seedling.

2. Correlation coefficients showing the relation between the serial numbers derived from the catalase activity and the serial number for the corresponding strains for either measure of vigor were not significant.

3. The relative position of inbred strains or the crosses between inbred strains of corn seedlings as determined by their catalase activity with reference to that of other strains and crosses appears to be fairly constant.

4. The order or rank of the leaf and stem tissue of a seedling when determined by the relative catalase activity was not always the same.

5. The seedlings grown in sand were on the average slightly more active than those grown in soil. The average activity of the leaf tissue was a little higher than that of the stem tissue of seedlings grown in either sand or soil. Apparently there was no parallel between the catalase activity of inbred strains of corn seedlings and that of the corresponding crosses.

The writer is indebted to Dr. O. F. Curtis for suggesting the problem as well as for advice and criticism in the interpretation of the data, to Dr. R. G. Wiggans for the seed without which the investigation would have been impossible and for figures showing the percentage the yield of the mature offspring was of the commercial stock, and to Dr. H. H. Love for help in determining the correlation coefficients.

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A PRELIMINARY STUDY OF SOME ENVIRONMENTAL FACTORS ON THE SPREAD OF CERCOSPORA LEAF SPOT AND YIELD IN CHECKED AND DRILLED SUGAR BEETS

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(Received for publication April 10, 1931)

Cercospora leaf spot of sugar beets caused by *Cercospora beticola* Sacc. is a disease which requires a relatively high humidity to develop in epidemic form. It seems logical, therefore, that any modification in the culture of the crop which would lower the humidity during the growing season should tend to reduce the amount of leaf spot. The elimination of crowding should permit greater circulation of air, and consequently lower humidity and a higher evaporation rate. This suggested measuring the effect on *Cercospora* development and yield of growing fewer plants per given area and adopting a uniform spacing comparable to checking of corn and other crops. If the wider spacing, through the checking system, showed a tendency to alleviate the leaf spot situation and maintain the existing returns per acre, more impetus would be given to much needed changes in the culture of the crop, resulting in better cultivation and marked reduction in hand labor. Data bearing on this problem accumulated during the growing seasons of 1929 and 1930 are recorded below.

PRELIMINARY EXPERIMENTS IN 1929

In order to test the relation of reduced humidity and a higher evaporation rate on leaf spot development and yield, preliminary experiments were carried out in 1929 in a field of beets belonging to the American Beet Sugar Company at Mason City, Iowa.[1] Four sets of plots were laid out 12 rows wide by one rod long with the rows 22 inches apart. The average stand of beets for the entire field was about 80 percent of a perfect stand. A perfect stand was considered as one in which the beets were spaced uniformly one foot apart in the rows. The sets of plots were thinned to average, one-half,

[1] The plot work was carried on in coöperation with the American Beet Sugar Company on their farm located at Mason City, Iowa, through the kindly interest of Mr. E. C. Moore, manager Mason City and Belmond plants.

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three-eighths, and one-fourth stand. These wide variations were chosen in order to afford large differences in environmental conditions in the respective plots.

The humidity as determined with a cog psychrometer was lowest in the one-fourth stand and highest in the average. The averages for the four plots were in order as follows: 59.9, 63.4, 65.6, and 67.1 percent. The soil moisture was likewise lowest in the one-fourth stand and highest in the average. The readings at 0–6 inches, 7–24 inches, and 25–36 inches were, respectively, as follows: one-fourth plot, 13.3, 12.13, 9.56; one-half plot, 13.58, 12.8, and 8.82; average stand plot, 14.38, 13.02, and 9.7 percent. The average rate of evaporation, as measured by Livingston porcelain bulb atmometers stationed among the foliage, was highest in the one-fourth stand and lowest in the average stand of beets. The rate between September 2 and 21 for the one-fourth-stand plot was 39.8 cc. per day and for the average stand plot, 25.2 cc. The leaf development was found by weighing leaf tracings cut from cardboard of uniform thickness. The percentages of increase for three of the plots between August 28 and October 7 were in the one-fourth stand 71.4; in the three-eighths 63.6; and in the average 33.3 percent. These data show that the growth response was more than doubled, the humidity was lowered, and the evaporation rate increased in the one-fourth-stand beets as compared with the average stand beets.

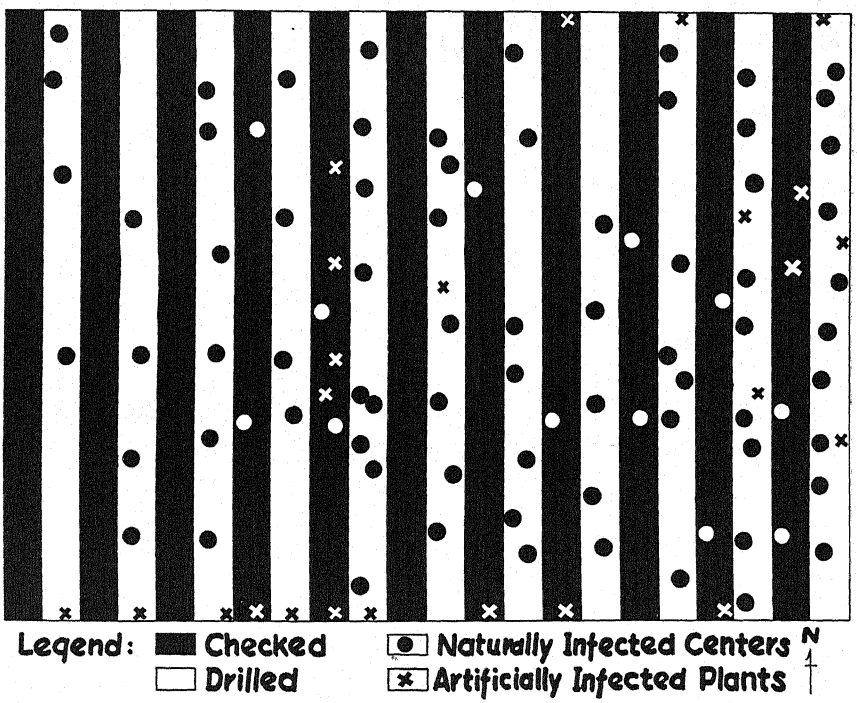
The year 1929 was not a bad one for leaf spot in Iowa, although the disease was more prevalent in July than usual. It was impossible to trace the development of leaf spot on individual plants in the 16 different plots, because some infection was already present when the experimental plots were laid out. However, it was observed that the amount of infection did not increase further during the growing season in the one-fourth-stand plots, while it did in the average.

The yields were obtained by harvesting and weighing the beets on each plot and calculating the average number of pounds per acre for each type of thinning. Due to soil variations, yields from only 6 plots were considered comparable. These are as follows: one-fourth stand 6,420 lbs., one-half 10,885, and average 14,755.

Although the data collected were in certain respects meager and in some cases contradictory, indications were that in practice it should be possible, without materially reducing the yield, to space the plants by checking so that environmental conditions would be more unfavorable to the development of leaf spot. This hypothesis was further substantiated through field observations reported to the authors by Mr. C. T. Lund, Supervising Agriculturist of the American Beet Sugar Company, from two field experiments made in 1929 on the comparative yield of checked and drilled beets. In both fields the checked beets yielded more than the drilled and entailed less hand labor.

Experiments Made in 1930

Following the leads gleaned from the 1929 experiments, more and larger plots, differently arranged and located, were laid out on a five-acre field in 1930. The twenty-two plots were arranged so that they consisted of strips one rod in width across the field. In 11 of these the beets were thinned to approximately 12 inches and in the other 11 to about 20 inches. The plots with different rates of thinning alternated. The plots were planted May 22 on good beet land that had been in corn the previous year, and were thinned between June 20 and 25. The checked beets were blocked with a two-row cultivator and each hill thinned to one plant. These were cultivated both ways three times beginning about two weeks after thinning and the drilled



Text Fig. 1. The spread of an artificial epidemic of Cercospora leaf spot on a field of sugar beets consisting of alternately checked and drilled beets in plots one rod in width. Note that the epidemic has spread north and west away from the artificial centers. A count will show that there are 6 times as many naturally occurring infection centers in the drilled plots, represented by the black discs, as in the checked plots, represented by the white.

plots received three cultivations one way. In addition to the cultivation, the drilled plots were hoed to keep them free of weeds.

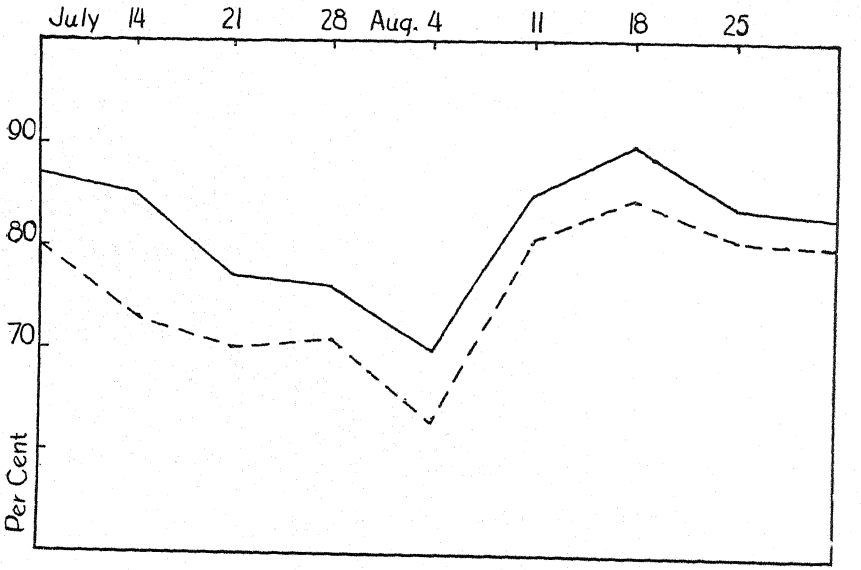
From time to time plants infected with *Cercospora* were placed in the margins of the plots as shown in text figure 1. The object was to follow the spread of infection. Between August 25 and 27 and September 8 and 12 the new infection centers were staked.

Environmental Factors in the Two Kinds of Plots

Before relating the amount of infection, tonnage, and sugar percentage, it may be well to state briefly the difference in the environmental factors in the different plots. The following factors were measured in the checked and drilled plots: air and soil temperature, humidity, light, wind, soil moisture, and evaporation.

The first three were compared by means of Friez and Sons weekly recording instruments, sunken in the ground between the rows in order to get the effect of shading, as early in the season as possible. These instruments were protected by regulation shelters and checked twice a week.

The differences in air temperature taken under cover of the beets were higher in the checked than in the drilled plots. The wider spacing permitted a greater amount of sunlight to penetrate into the rows, thus raising the temperature above that of the more closely planted beets. Since there is an inverse ratio between air temperature and relative humidity, the increased air temperature in the checked beets lowered the relative humidity in these plots in the day time. The maximum daily soil temperature at a depth of three inches ran as much as ten degrees higher in the checked than in the



Text Fig. 2. Average daily humidity by weeks in drilled beets (solid line) and in checked (broken line). The average humidity difference during the season is 6 percent.

drilled plots, whereas the night temperature ran approximately the same in both.

The hygrograph records show a marked daily decrease in the relative humidity of the checked over the drilled plots. Text figure 2 shows the combined day and night averages of readings taken from the charts at two-

hour intervals. Graphs based on readings for the hours of the day, omitting the night, or on the differences between high and low readings rather than on the average of two-hour readings for the day and night, show a wider variation for the two plots. The average period of 100 percent night humidity from July 14 to September 21 for the drilled plots was 9.4 hours and for the checked 7.1 hours, an average difference of 2.3 hours. This reduction in the checked beets of the time during which the air was saturated on humid nights may be the factor which reduced infection in these plots. However, the daily minimum humidity, which is three to ten percent lower in the checked beets, seems to be an important factor.

The soil moisture remained about the same in the two types of plots except during a critical period at 12–24-inch depths. The amount of available water is shown in table 1. The percentage of moisture at permanent wilting from tests of a mixture of soil at all depths was 12.47 percent. Since the number of beets growing on the same area was greater in the drilled plots, it would seem that there was less water per beet than for the individuals in the checked plots. It may be that this reduction in the amount of water per beet is reflected in the reduced rate of growth of roots and a decrease in final yield.

TABLE 1. *Available Soil Moisture at Different Depths (i.e. Percent Above the Moisture Equivalent) in Drilled and Checked Plots*

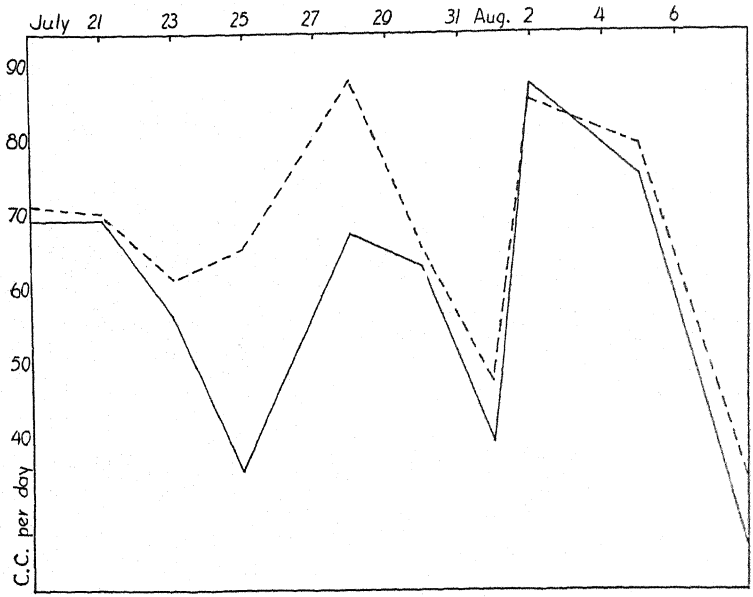
Date	Soil Moisture							
	0–6 Inches		6–12 Inches		12–24 Inches		24–36 Inches	
	Checked	Drilled	Checked	Drilled	Checked	Drilled	Checked	Drilled
July 12	13.6	9.2	8.5	7.6	10.6	8.6	7.6	8.6
July 22	7.6	7.6	6.6	5.6	7.1	6.6	7.6	7.6
July 29	8.6	10.6	6.6	7.6	8.6	6.6	6.6	6.6
Aug. 5	15.6	16.6	10.6	10.6	8.6	4.6	4.6	4.6
Aug. 25	5.6	2.6	8.1	5.6	9.1	1.6	5.6	2.6
Sept. 9	1.3	0.9	1.3	0.9	0.0	1.6	1.6	1.6
Sept. 17	12.2	12.6	2.1	2.6	2.1	0.6	1.6	0.6

Evaporation was measured by means of Livingston porcelain atmometer cups, with rain-correcting set-ups. These showed an average daily increase in the checked plots over drilled of 3.6 cc. Text figure 3 shows the most important increase in the checked beets of atmometers set between the plants in the rows.

Wind velocity was measured in feet pet minute by means of Tycos hand anemometers. There was a marked increase in the rate of wind movement in the checked beets. This results in increased evaporation from the soil and transpiration from the beets which may inhibit the development of *Cercospora beticola*. Text figure 4 shows the difference in wind velocity in the two plots.

Cercospora Leaf Spot Development on the Plots

It is important to record the leaf spot development on the different plots First it should be said that leaf spot did not develop in epidemic form in Iowa in 1930. The loss was normal except in a few fields. On the other

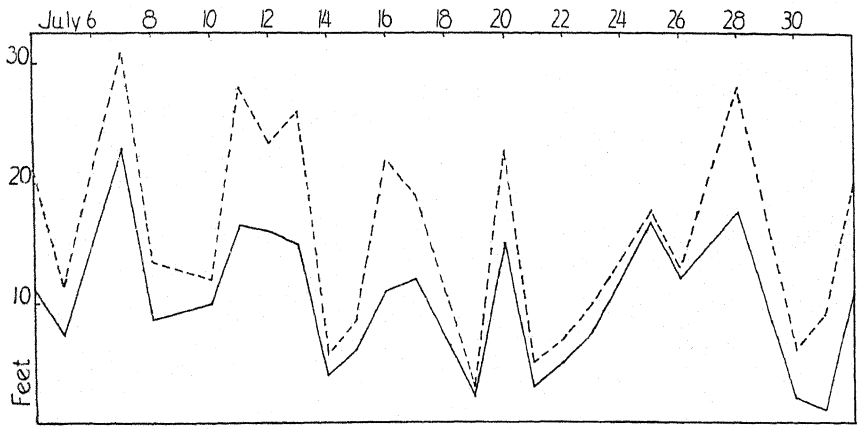


Text Fig. 3. Evaporation in cc. per day from atmometers set between the plants in rows of drilled beets (solid line) and in the checked (broken line). As in the humidity and wind graphs, the greatest variation between the two plots occurred in July during the critical period of leaf spot development.

hand, enough primary infection was present to develop into epidemic form had more favorable weather occurred. The fact that Cercospora leaf spot developed slowly facilitated following its spread. The results obtained in the experiment in the spread of Cercospora leaf spot are shown in table 2. The plot was exposed to artificial infection by establishing infected sugar beet plants about the plots. By August 27, all of the artificially inoculated transplants had become definite infection centers and were staked. In addition to the artificially infected centers, 17 new centers had developed naturally in the drilled plots and two in the checked. These were also staked as shown in text figure 1. The plots were numbered from east to west and the artificially infected plants were established along the eastern and southern margin of the field. By August 27 the infection had not spread naturally beyond plot No. 7.

By September 12 the number of naturally occurring infection centers had increased from 17 to 72 in the drilled plots and from 2 to 12 in the checked. In other words there were six times as many in the drilled as in

the checked plots. By that time the infection had spread across the field. The results obtained in this experiment indicate that the conditions for the development of Cercospora leaf spot were less favorable in the checked than in the drilled beets.



TEXT FIG. 4. The average daily wind velocity in feet per minute between the beets in drilled plots (solid line) and in the checked (broken line). The average velocity during July was 16 feet per minute in the checked plots compared to 9.2 feet in the drilled. At periods during the month the wind velocity was almost doubled in the checked beets. The desiccating effect of the wind during these intervals no doubt contributed to the reduction of leaf spot.

A COMPARISON OF THE RATE OF MANUFACTURE OF FOOD AND YIELD IN THE CHECKED AND THE DRILLED PLOTS

Having measured the Cercospora leaf spot spread in relation to the two systems of spacing, attention was turned to the effect on the returns per acre: i.e. the comparative tonnage, percent sugar, and purity. As already indicated, a decrease in leaf spot injury would be of little value if the crop returns were appreciably lower. Likewise the reduced cost of production forseen by the checking system might be nullified by the comparative yields of the two systems. The significance of this phase of the problem led to initiating an adequate plan for obtaining tonnage, percent sugar, and purity on all the plots in 1930.

Major quadrats two meters on a side and four square meters in area were harvested from each plot to measure the yield, percentage of sugar, and purity. One series of such quadrats was harvested from each end of the field and a third taken from the center, or a total of 33 quadrats were harvested from each series of checked and drilled plots.

In this connection some of the growth-controlling factors were measured, as light intensity and its effect on rate of photosynthesis. The light intensity in each plot was compared every two weeks by means of the Clements

TABLE 2. *The Induced Spread of Cercospora Leaf Spot into the Checked and Drilled Plots as Determined on August 27 and September 12, 1930*

Plot No.	No. Plants Inoculated on			Total Number Infection Centers			
				August 27		September 12	
	June 30	July 24	August 5	Checked	Drilled	Checked	Drilled
1*	3				6		20
2		2		2		4	
3		2			7		8
4		1		1		3	
5			1		3		7
6				1		2	
7					4		4
8		2		3		3	
9					0		
10	1			1		2	
11			1		4		8
12				0		0	
13		1			1		10
14		2	3	5		7	
15			1		1		5
16			1	1		3	
17			1		1		7
18				0		0	
19			1		1		2
20				0		0	
21	1				1		5
22				0		0	
			Total	2	17	12	72

* The odd numbers denote drilled, and the even checked, plots.

photometer. Table 3 shows that the checked beets have intercepted less light due to wider spacing. Their greater rapidity of growth was in a large measure due to the ability of the lower banks of leaves to work at a high degree of efficiency.

The growth of the beets was determined in the following manner: Records were made of the height, width, and number of leaves, and the diameter of

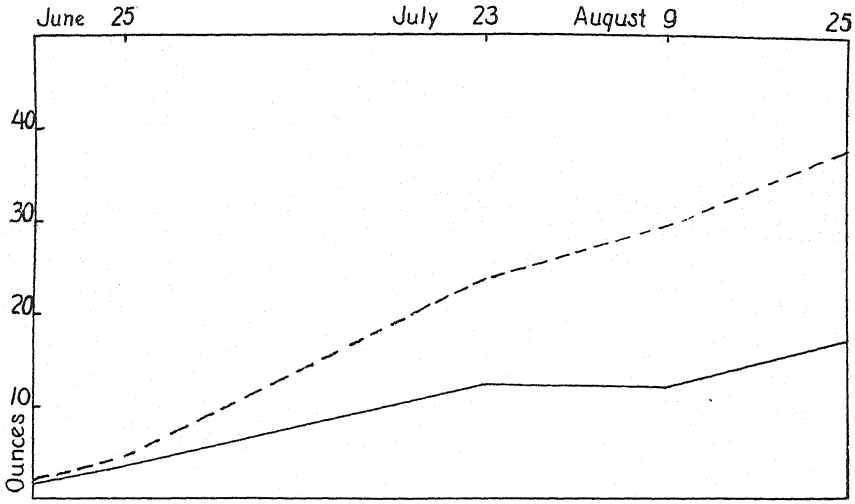
TABLE 3. *Light Intensity in Percentage of Full Sunlight Under the Leaves in the Rows of Checked and Drilled Beets*

Date	Time, A.M.	Checked, Percent	Drilled, Percent
July 18	10	25	20
July 23	8	13	6.6
Aug. 1	2:30*	17	15
Aug. 20	10:30	14	5
Sept. 1	10:30	12	5
Sept. 15	10:30	12	6
Sept. 21	10:30	14	7

* P.M.

the roots at the crown, results of which are shown in table 4. The beets were dug at two-week intervals and weights taken. Text figure 5 shows the increase in green weight during the season.

These measurements and weights show that the checked beets made a steady growth throughout the entire season, while the drilled beets made no



TEXT FIG. 5. Average green weight of beet roots throughout the early part of the growing season in drilled beets (solid line) and in checked (broken line). The checked beets made a steady increase in weight during the entire season while the drilled beets made practically no growth following the drought in mid season. The drilled beets were twice the weight of the checked beets at the beginning of the dry period.

growth during the dry part of the summer. The checked beets averaged one and one-half pounds on July 23, while the drilled beets remained small until after the late summer rains.

The rate of photosynthesis was determined by the picramic method. Fifty leaf punches of one square centimeter each were taken from ten plants in each plot. The first of these were taken before eight in the morning, and

TABLE 4. *Measurements of Leaves and Roots in the Drilled and Checked Plots*

Date	Spacing	Height Leaves, Inches	Width Leaves, Inches	Number Leaves	Diameter Root Crown, Inches
July 23.....	Drilled	18	5.7	15	1.5
	Checked	18	6	18	1.6
Aug. 1.....	Drilled	20.7	7	19	2.3
	Checked	20.1	7	20	2.5
Aug. 9.....	Drilled	23	7	19	2.8
	Checked	25	7	22	3.5
Aug. 23.....	Drilled	23	7	19	3
	Checked	25	7	25	4.3

the same number from the same leaves again at 4:15 in the afternoon on the same day. The difference in sugar content of these was interpreted as an index of the rate of photosynthesis for that day. The rate in checked and drilled plots was nearly the same on young and mature leaves, but the lower leaves of the checked plants were very much more efficient. Values obtained do not represent the total amount of photosynthate because of active translocation during the day.

The entire leaf surface of five checked and five drilled beets was determined, and tabulated in three classes: young, mature, and old leaves. Young leaves were those not of full size and still perfectly upright. Mature leaves were those fully developed and still distinctly bright. Old leaves were those that were down under the mature leaves (often quite pale or yellow in the drilled plots). The total surface was multiplied by the rate of photosynthesis determined for each plot and from this the total day's work for the whole plant computed. These values were obtained for the checked and drilled plots, respectively: Young leaves, 4.0671 and 1.0968 grams; mature, 6.0914 and 3.0751; old, 7.0624 and 0.8845. The average per plant in the checked plot was 3.4442, and in the drilled 1.0113, grams. Greater leaf surface of the young and mature leaves and better exposure to light of the old leaves in the checked plots probably were responsible for the greater growth.

Results obtained from the checked and drilled plots are shown in table 5. The checked plots yielded an average of 2219 pounds of beets and 20

TABLE 5. *Yield in Checked and Drilled Sugar Beets*

Spacing	Computed Averages					
	Weight per Plot	Number Beets per Plot	Percent Sugar	Purity	Pounds Beets per Acre	Sugar per Acre
Checked.....	30.43	17	14.56	82.5	31,029	4528
Drilled.....	28.49	27	15.61	80.5	28,810	4508

pounds of sugar per acre more than the drilled. Although these differences are not sufficiently large to be significant when submitted to statistical analysis, they do tend to show that the yield may not fall much below the drilled. It is probably equally important that the differences in purity are in favor of the checked beets. As is well known, this facilitates the processing.

The average weight per beet of the checked beets was increased over 40 percent which more than offset the higher sugar percentage of the drilled beets. In this connection it is important to record that Mr. C. T. Lund, General Agriculturist for the American Beet Sugar Company, reported to the authors that he had under his supervision over 10,000 acres of checked beets and that the yield was at least equal to that of the drilled and the labor less.

The Effect of Doubles on Yield

In order to test the influence of doubles (two beets in a place) on the yield, a series of plots was laid out in which the rate of thinning was varied. The drilled beets were thinned to approximately 12 inches and the checked ones to 20 inches. The checked beets were located as follows: check, 1-1-1; two beets every other place, 1-2-1; and two beets every third place, 1-1-2. The plots were replicated four times. The yield was ascertained by three yield blocks from each plot making a total of 12 for each replication. It is evident that under the conditions of this experiment two beets in a place did not reduce yield per acre. The beets planted 1-1-2 yielded the heaviest. The rows were 22 inches apart so that the beets were 22 × 20 inches. Table 6 shows the yield results.

Table 6. *The Influence on Yield Due to Rate of Thinning in Checked Beets*

Rate Stand	Computed Average					
	Number Beets per Plot	Weight Beets per Plot	Percent Sugar	Purity	Pounds Beets per Acre	Pounds Sugar per Acre
Drilled.....	29	29.4	16.09	83.9	29,745	4,775
Checked, 1-2-1....	20	32.8	15.6	83.8	33,184	5,176
Checked, 1-1-2....	18	33.29	15.58	83.2	33,679	5,247
Checked, 1-1-1....	16	32.41	15.16	83.1	32,788	4,953

Summary

Environmental conditions, spread of Cercospora leaf spot, rate of growth, and yield were studied during 1929 and 1930 in checked and drilled sugar beets.

The average humidity was three to ten percent lower from July 14 to September 21 in the checked than in the drilled plots. The average rate of evaporation and wind velocity, on the other hand, were increased 3.6 cc. and 10 feet per minute, respectively, in the checked plots.

Cercospora leaf spot did not develop in epidemic form in either 1929 or 1930. In 1929 less leaf spot developed in the thinned plots than in the unthinned. Infection centers did develop in July and August generally throughout the beet growing district in Iowa. In 1930 when checked and drilled beets were exposed to artificial infection, six times as many centers of infection developed in the drilled as in the checked.

The tonnage, percent sugar, purity, and total pounds of sugar per acre were as follows: checked plots, 31,029, 14.56, 82.5, and 4528; and drilled plots, 28,810, 15.61, 80.5, and 4508, respectively.

Light intensity was nearly twice as great under the checked beets as the drilled. The growth rate showed a steady increase in green weight for the checked as compared to a much slower and varied growth made by the drilled beets.

The rate of photosynthesis indicated that for the young and mature leaves the rate per square meter of leaf area in the drilled and checked beets was approximately the same, but the old leaves on the checked plants were much more efficient.

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THE INFLUENCE OF LIGHT AND TEMPERATURE UPON THE UTILIZATION BY YOUNG SEEDLINGS OF ORGANIC RESERVES IN THE SEED

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The first year is the most critical period in the life of a forest tree seedling. The initial impetus, imparted to the seedling from the stored materials in the seed, determines to a large extent whether the seedling will become established or perish. Light and temperature are important factors in determining how rapidly the seedling draws upon its food reserves, and both of these in any forest region are more or less directly dependent upon the density of the canopy. The effect of light and temperature upon the growth of seedlings during this dependent stage is here treated separately from their effect upon the establishment and subsequent growth of seedlings.

The native conifers of the Lake States region have small seeds, which are comparatively slow and irregular in germination. The difficulties involved in weighing and handling individually such small seeds and seedlings rendered it inadvisable to use these conifers for preliminary experiments, and hence herbaceous seedlings were used, it being assumed that the fundamental principles established would probably hold for all plants.

Radiant energy, particularly that emitted by the sun, plays a very important rôle in the growth and metabolism of both plants and animals. Light is necessary for photosynthesis in higher plants and is also essential for many other chemical and physiological processes which occur in plant tissue. The question sometimes arises as to whether the higher plants grow with equal rapidity with or without light provided they have an ample supply of reserve foods to draw upon. The fact that many plants, such as willow shoots, date palms, maize, and others grow in length mostly at night (Probst, 4; Mason, 3) has led some workers to suppose that light inhibits plant growth. On the other hand, when oak seedlings were grown under various degrees of shading it was observed that those plants which received the most light appeared to draw most heavily upon the seed for nourishment.

To obtain further evidence relative to this question a series of four experiments were carried out with maize seedlings to determine whether light does accelerate the utilization of their food reserves. The results of these experiments indicate that when the seedlings in the light and in the dark are grown with the same soil temperatures no such acceleration can be detected. If either those in the light or those in the dark are maintained at a higher soil temperature, the seedlings having the higher temperature utilize the stored material in the seed faster.

LITERATURE

After the work was well underway a paper by Lubimenko and Karisnev (2) on the same subject came to the writer's attention, from which tables 1 and 2 are taken.

TABLE 1. *Effect of Shading upon the Growth and Utilization of Stored Materials by the Barley Seedlings. Percentage Weights are Based on Initial Seed Weight*

Intensity of Illumination	December 24–January 16, 7°–11° C.				February 1–19, 11–13° C.			
	Weight of Plant in mgs.	Weight of Plant	Weight of Residual Seed	Weight Lost	Weight of Plant in mgs.	Weight of Plant	Weight of Residual Seed	Weight Lost
		In Percent of Initial Seed				In Percent of Initial Seed		
Full light of day	11.4	38.7	9.8	51.5	15.0	51.0	11.9	38.1
Daylight weakened by one layer of paper	13.8	47.0	12.2	40.8	13.6	46.2	11.2	42.6
Daylight weakened by 2 layers of paper	14.7	50.0	10.2	39.8	14.5	49.3	10.9	39.8
Daylight weakened by 4 layers of paper	14.1	48.0	10.8	41.2	14.4	49.0	11.9	39.1
Darkness	12.6	42.8	12.9	44.3	13.3	45.2	11.9	42.9

TABLE 2. *Effect of Shading upon the Growth and Utilization of Stored Materials by the Wheat Seedling in Air with and without Carbon Dioxid. Temperatures 12°–15° C. Grown March 17–30*

Intensity of Illumination	Ordinary Air				Air Without Carbon Dioxid			
	Weight of Plant in mgs.	Weight of Plant	Weight of Residual Seed	Weight Lost	Weight of Plant in mgs.	Weight of Plant	Weight of Residual Seed	Weight Lost
		In Percent of Initial Seed				In Percent of Initial Seed		
Full light of day	20.6	46.5	15.1	38.4	—	—	—	—
Daylight weakened by one layer of paper	20.5	46.3	11.5	42.2	17.8	40.2	14.1	44.7
Daylight weakened by 2 layers of paper	25.3	57.3	13.5	29.2	21.6	48.8	20.0	31.2
Daylight weakened by 4 layers of paper	21.3	48.1	12.9	39.0	18.8	42.5	15.8	41.7
Darkness	18.3	41.3	11.9	46.8	17.9	40.6	12.2	47.2

These workers grew wheat and barley in nutrient solution placed in glass jars of 5 to 6 liters capacity. The jars, freely exposed and covered with one, two, and four layers of white paper and by a layer of black paper, were placed in a window. The plants were grown in Leningrad during the winter and early spring. In one experiment the plants were grown in the absence

of carbon dioxid. The authors point out that maximum dry weight of the plants occurred with one exception in those shaded by two layers of paper, which they considered to be optimum illumination. The wheat plants grown without carbon dioxid showed the same general trends as those grown with carbon dioxid. The weight lost through respiration shows an opposite trend to the weight of the seedling. By microchemical methods the authors found that the minimum amount of soluble carbohydrates occurred in those plants having minimum rate of respiration and maximum dry weight. They state that it cannot be doubted that light produces a direct action on the accumulation of dry material in plants during their purely saprophytic nutrition.

METHODS

Rustler maize, a variety of *Zea Mays* commonly sown in Minnesota, was used in all experiments. The grains used were carefully selected for uniformity of weight and shape and weighed individually before planting. Five samples of 25 seeds each were selected from the same lot and dried in the oven to determine initial moisture content. The seed was sterilized with 0.1 percent Semesan for two hours at 47° C. and soaked overnight in distilled water before planting. The plants were grown in a rich sandy loam in 4-inch clay pots. In the first two experiments only one grain was sown in a pot. To economize in space two seeds were sown per pot in the third and five in the fourth experiment. In every case individual records were kept for each seed. Seeds slow in germinating, infected with fungus, or otherwise abnormal, were not used in the final analysis.

The plants were harvested in lots of ten. The soil was carefully removed from the roots by washing and the seedling separated from the seed at the scutellum. Residual seed and seedling were dried and weighed and the results expressed in percentage of the initial dry seed weight.

RESULTS

First Series. Greenhouse, December 17, 1929–January 11, 1930

After sowing, the pots were placed in the greenhouse, one set on top of the bench and the other underneath. The greenhouse was equipped with a unit heater system which provided for good air circulation. Both air and soil temperature were, nevertheless, 3°–6° C. higher above the bench, especially during sunny days. Germination occurred about two days earlier for the plants in the light, and subsequent growth was more rapid. Those under the bench were yellowish green in color and decidedly etiolated. The results, shown in table 3, indicate that the plants in the light utilized the seed reserves much faster, but whether the difference was caused by light or by temperature can not be decided.

TABLE 3. *Effect of Light on Utilization of Stored Material by Maize Seedlings Grown in a Greenhouse December 17, 1929–January 11, 1930*

Days from Planting	No. Plants Used		Percent of Initial Seed Recovered	
	Light	Dark	Light	Dark
17	10	10	31.1	50
18	10	10	22.8	43.4
20	20	20	16.0	36.7
21	30	20	14.4	28.9
22	10	10	14.2	32.8
23	5	10	13.2	33.4
24	5	10	14.5	29.9
25	5	5	14.0	26.5

Second Series. 20° Temperature Room, January 17–February 6, 1930

An attempt to eliminate the temperature factor was made by growing the plants in a constant-temperature room, artificially cooled. The plants in the light were placed on a table 5 feet beneath a 500-watt incandescent lamp. The others were placed beneath the table. The plants on the table received 70–200 foot-candles illumination and those beneath, 3 foot-candles. The lamp was burned continuously. An electric fan was used to provide uniform air conditions. Air temperatures were practically the same in the light and dark but soil temperatures were 1°–4° C. higher in the light. Pots directly under the lamp averaged 0.5°–2° C. higher than those farther from the lamp. The results are shown in table 4.

TABLE 4. *Effect of Light upon the Growth and Utilization of the Seed by Maize Seedlings, Placed in a 20° C. Constant-temperature Room, January 17–February 6, 1930. All Percentages Are Based on Dry Weights*

Days from Planting	No. Plants Used	Light			Dark		
		Percent of Initial Seed Weight			Percent of Initial Seed Weight		
		Residual Seed	Seedling	Seed Plus Seedling	Residual Seed	Seedling	Seed Plus Seedling
12	10	28.8	40.6	69.6	48.6	27.7	76.3
13	10	32.1	40.1	72.2	48.9	27.1	76.0
14	10	29.2	38.5	67.7	35.1	33.7	68.8
15	10	20.9	50.6	71.5	34.7	33.9	68.6
16	10	20.1	56.7	76.8	30.5	37.4	68.0
18	10	15.4	56.7	72.1	29.8	34.7	64.6
19	10	14.5	59.8	74.4	19.1	41.3	60.4
20	10	13.6	63.8	77.4	22.9	38.8	62.7
21	10	12.4	74.6	87.0	13.4	43.2	56.6

The plants in the light had a higher rate of respiration than those in the dark, as can be seen by noting the combined weight of seed plus seedling. During the first few days, before the influence of photosynthesis became

prominent, the combined weight was greater in the plants grown in the dark. It is seen that the plants in the light used the seed reserves faster than those in the dark and also accumulated dry matter more rapidly. Those directly under the lamp used the reserves faster and grew faster than those farther from the lamp, as shown in table 5. Again it was felt that the difference could not be attributed to light without more precise temperature control.

TABLE 5. *Difference between Plants under Lamp and Those at One Side in Growth and Utilization of the Seed. The Mean Weights for all Seedlings Sampled Each Day is Used as a Basis for Calculating Percentages. 20° C. Temperature Room*

Days from Planting	97 Foot-candles				68 Foot-candles			
	Percent of Mean				Percent of Mean			
	No. Plants	Wt. of Seed	Wt. of Seedling	Seed Plus Seedling	No. Plants	Wt. of Seed	Wt. of Seedling	Seed Plus Seedling
12.....	5	90	100	95	2	101	118	102
13.....	3	95	100	101	4	105	98	97
14.....	2	108	90	98	5	105	97	102
15.....	5	87	109	103	5	111	99	97
16.....	5	72	122	107	2	149	71	91
18.....	4	90	112	107	3	96	91	99
19.....	2	86	117	111	5	103	98	98
20.....	3	86	103	99	3	113	90	99
21.....	6	106	100	101	3	86	103	103
Mean	35 8+, 27−	91	106	102	32 6−, 26+	109	96	99

Third Series. February 19–March 11, 1930

To eliminate soil temperature as a factor a third series was run in a large room subjected to rough temperature control. The plants in the light were placed about 5½ feet beneath a 1000-watt lamp, while those in the dark were placed beneath an open coil type 550-watt hot plate. Lamp, heater, and an electric fan were operated continuously. Air temperatures averaged 24.6°–26.1° C. in the light and 25.3°–26.7° C. in the dark. Soil temperatures averaged 23.5°–25.8° C. in the light and 23.8°–25.1° C. in the dark. The temperatures in adjacent pots showed considerable variation depending upon the wetness of the surface layer of soil. Measurements with a glass enclosed thermopile (6) showed the radiation intensity in the dark to be about 50 percent of that in the light. The illumination was 104 foot-candles and 0.4 foot-candles. During the first few days after germination, the plants in the dark were deliberately maintained at a slightly higher temperature than those in the light. The results, given in table 6, show a more rapid utilization of stored materials in the seeds in the dark during the first few days and subsequently no significant differences.

TABLE 6. *Effect of Light upon the Growth and Utilization of Stored Materials by the Maize Seedling. Third Series. February 19–March 11, 1930*

Days from Planting	No. Plants Used	Light			Dark		
		Percent of Initial Seed Weight			Percent of Initial Seed Weight		
		Residual Seed	Seedling	Seed Plus Seedling	Residual Seed	Seedling	Seed Plus Seedling
10.....	10	43.6	32.8	76.4	33.4	41.3	74.7
11.....	10	36.7	39.3	76.0	31.9	40.0	71.9
13.....	10	19.6	53.1	72.7	16.0	50.2	66.2
14.....	10	18.6	50.6	69.2	14.9	47.1	62.0
15.....	10	14.9	59.5	74.4	14.8	46.6	61.3
16.....	10	14.4	63.9	78.3	13.9	45.1	61.1
17.....	10	13.2	61.0	74.2	11.7	47.8	59.6
18.....	10	12.7	64.2	76.9	12.3	47.0	59.3
20.....	10	10.3	73.3	83.6	11.3	44.1	55.4
21.....	10	10.5	69.6	80.1	11.2	42.3	53.5

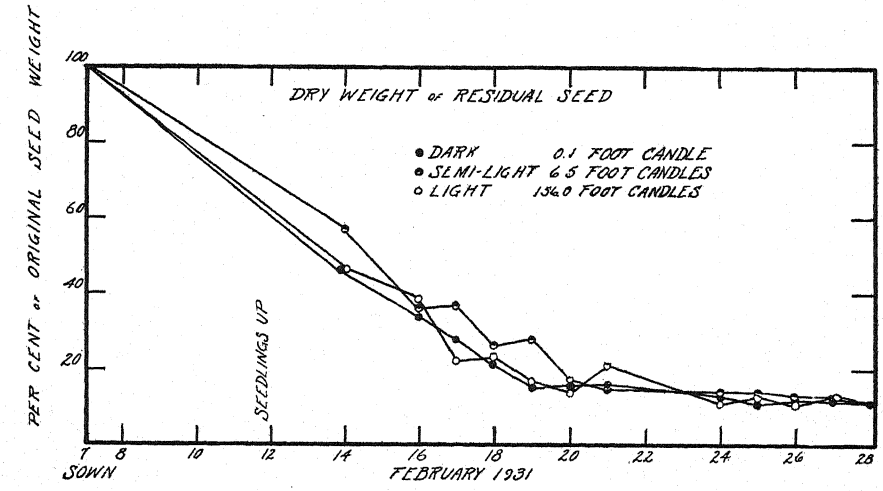
Fourth Series. February 7–28, 1931

This series consisted of three groups of 125 seedlings each. One was placed in the dark and irradiated with a 550-watt hot plate, a second was placed directly under a 1000-watt lamp at about 4½ feet distance, and a third was placed at one side of the lamp so as to receive intermediate illumination. To eliminate temperature changes due to the wetness of the surface soil the seed sown in pots was covered with a ½ inch layer of fine sand. The pots were placed in large trays and irrigated from below. Only rarely did the surface sand become moist, so temperature differences were very slight between adjacent pots. Soil temperatures were read every half hour during the day for the first two weeks and at hourly intervals thereafter. At each reading adjustments were made to equalize temperatures. The lamp and hot plate were burned for 12 hours each day and an electric fan ran continuously. The daily temperatures and running mean up to the close of each day are given in table 7. Except for the last two days the soil temperature averaged 0.1°–0.3° C. higher in the light than in the dark, while in the moderately lighted group the temperature was about 1.5° C. lower than in the best lighted group. The light, as measured with the Macbeth Illuminometer, was about 156, 65, and 0.1 foot-candles for the three groups. A glass covered thermopile (6) gave values of 0.04, 0.02, and 0.01 calories per square centimeter per minute while an open thermopile gave relative readings of 12, 8, and 10 for the strong light, intermediate light, and dark groups, respectively.

The results are shown in table 8 and in text figures 1, 2, and 3. Examination of text figure 1 shows no significant difference between the plants in strong light and those in the dark. It shows a very decided difference, however, between those in weak light and the other two groups until the residual seed was practically exhausted. This difference must, therefore,

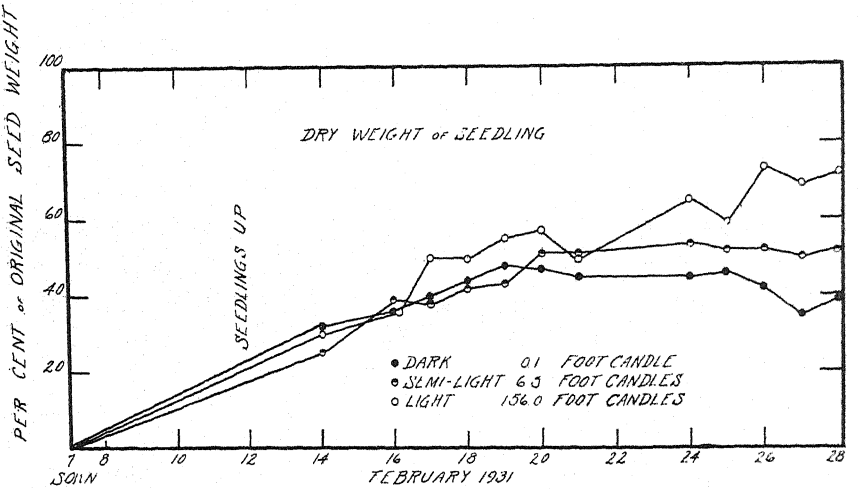
TABLE 7. *Fourth Series. February 7–28, 1931. Daily Mean and Running Mean Soil Temperatures under Different Light Conditions*

Date	No. of Readings Taken	Under Lamp 156 F.C.		Side of Lamp 65 F.C.		In Dark 0.1 F.C.	
		Daily Mean	Mean to Date	Daily Mean	Mean to Date	Daily Mean	Mean to Date
February							
7.....				Seed sown			
9.....	16	21.1	21.1	21.7	21.7	22.0	22.0
10.....	18	22.0	21.6	20.0	20.8	22.0	22.0
11.....	18	24.5	22.5	21.7	21.1	24.3	22.8
12.....	9	24.5	23.0	21.9	21.3	23.0	22.8
13.....	18	23.7	23.2	21.8	21.4	23.1	22.9
14.....	12	22.0	23.0	21.2	21.4	21.6	22.7
15.....	13	23.9	23.1	22.3	21.5	23.8	22.8
16.....	11	24.3	23.3	23.3	21.7	24.1	23.0
17.....	10	23.4	23.3	22.3	21.8	23.7	23.1
18.....	11	21.7	23.1	21.2	21.7	22.4	23.0
19.....	12	23.9	23.2	22.2	21.8	24.3	23.1
20.....	10	22.6	23.1	21.8	21.8	22.2	23.0
21.....	8	23.1	23.1	21.2	21.8	24.1	23.1
22.....	7	23.6	23.1	21.6	21.8	22.3	23.0
23.....	8	26.1	23.4	22.9	21.8	24.1	23.1
24.....	11	24.4	23.4	23.5	21.9	24.1	23.2
25.....	10	24.2	23.4	22.3	21.9	22.4	23.1
26.....	7	24.7	23.5	21.9	21.9	21.3	23.0
27.....	8	30.2	23.8	27.0	22.2	25.4	23.2



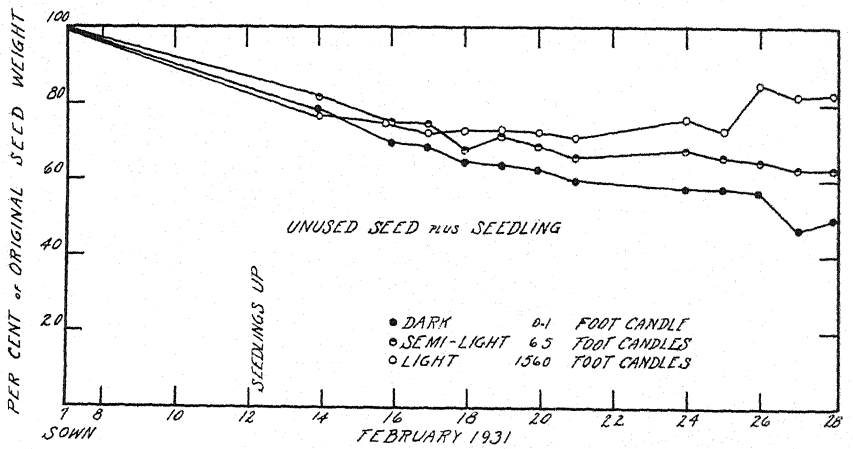
TEXT FIG. 1. Dry weights of residual maize seeds grown in two intensities of light and in darkness. Note the insignificant differences between those in strong light and those in the dark.

be attributed not to light but to temperature. Up until February 20 the plants in the dark weighed more than those in weak light. After this date the products of photosynthesis evidently became of importance in the nutrition of the plants in subdued light, as henceforth the plants in the dark



TEXT FIG. 2. Growth of maize seedlings in three light conditions.

decreased in weight while those in the light continued to increase. In text figure 3 the combined weight of seed and seedling is plotted. Except for the first lot sampled the plants in the dark showed the greatest weight lost.



TEXT FIG. 3. Percentage of original seed recovered on various dates from maize plantings under three light conditions.

TABLE 8. *Dry Weights of Residual Seeds and Seedlings after Growth under Different Light Conditions. Fourth Series*

Date	Under Lamp, 156 F.C.				Side of Lamp, 65 F.C.				Dark, 0.1 F.C.			
	No. Plants Used	Percent of Initial Seed Weight			No. Plants Used	Percent of Initial Seed Weight			No. Plants Used	Percent of Initial Seed Weight		
		Residual Seed	Seedling	Seed Plus Seedling		Residual Seed	Seedling	Seed Plus Seedling		Residual Seed	Seedling	Seed Plus Seedling
February												
12.....						Seedlings up						
14.....	10	46.8	30.1	76.9	10	56.8	25.1	81.9	10	46.7	32.6	79.3
16.....	10	38.7	36.1	74.8	10	35.8	39.1	74.9	10	34.4	36.0	70.4
17.....	10	22.7	49.9	72.6	10	37.1	37.8	74.9	10	28.5	40.6	69.1
18.....	10	23.8	49.8	73.6	10	26.2	42.0	68.2	10	21.5	43.8	65.3
19.....	9	17.3	55.7	73.0	8	28.4	43.7	72.1	8	15.9	48.3	64.2
20.....	7	14.6	57.9	72.5	9	17.2	51.9	69.1	10	16.1	47.2	63.3
21.....	7	21.3	49.5	70.9	8	15.0	51.1	66.1	8	16.7	45.0	60.5
24.....	9	11.0	65.3	76.3	8	14.4	53.7	68.1	8	13.6	44.9	58.5
25.....	10	13.7	59.0	72.7	10	14.1	52.1	66.2	8	11.3	46.4	57.8
26.....	7	11.0	73.7	84.7	10	13.3	51.7	65.0	9	11.8	42.2	57.1
27.....	7	12.9	68.8	81.7	8	13.0	50.0	63.0	8	12.3	35.3	47.6
28.....	7	10.7	71.7	82.5	10	10.9	51.7	62.7	9	11.7	39.2	50.9

DISCUSSION

If light has any effect on the rate of metabolism of the maize seedling aside from its indirect effect in increasing temperature, the effect is too slight to be detected by the means employed in this experiment. The writer is fully aware that infra-red might have an accelerating effect which would not have been detected since both plants in the light and those in the dark received abundant infra-red radiation. It is also possible that sunlight might produce an acceleration of metabolism which would not be noticeable when artificial light is employed.

The influence of temperature on plant metabolism is well illustrated by these experiments. Even in cases where control between two series is as close as one degree centigrade, temperature may still be a disturbing factor of sufficient importance to vitiate results. In this connection it is interesting to consider the work of Lehenbauer (1) who found that an increase in temperature from 22° C. to 23° C. caused an increase in the hourly rate of elongation of maize shoots from 0.59 to 0.64 mm. or 11 percent, when the observation period extended over 12 hours. In the fourth series, until February 19th, the temperatures were 23.1° C. and 21.7° C. for the strong light and weak light, respectively. The corresponding dry weights of seedlings were 55.7 and 43.7 percent of the initial seed. Lehenbauer's table (1, page 276) would give an increase in elongation for the plants in strong light of about 14 percent in this temperature range while the increase in dry weight of the entire seedling was actually 27 percent. The additional 13

percent is probably due in large part to the increase in photosynthetic activity caused by increased illumination, and also to the different response to temperature when measured as dry weight rather than as elongation. Those in the dark at 23.0° C. weighed 10.5 percent more than those in subdued light at 21.7° C., while the increase to be expected according to Lehenbauer's table is 12.3 percent. The products of photosynthesis, added to those in the light, could easily account for the 1.8 percent difference between actual and expected values. Beyond February 19 the products of photosynthesis were sufficient to provide for the greater part of the seedling nutrition. It was not until the sixth day after the seedlings came up, or February 17, that the seedlings in full light attained a greater dry weight than those in the dark, regardless of a slightly higher temperature in the light; but by this time the photosynthetic process must certainly have been sufficiently active to cause a material increase in the weight of the seedling. Neither those in subdued light nor those in strong light showed a significant increase in dry weight over those in the dark until photosynthetic activity became a prominent factor.

We have, therefore, no conclusive evidence that light produces a measurable acceleration, either in the rate of utilization of stored products in the maize seed, or in the dry weight of the seedling, aside from its effect on photosynthesis and temperature.

This work with maize seedlings fails to confirm the conclusions of Lubimenko and Karisnev (2) cited above. The conditions of experimentation were decidedly different as to plant material used, temperature, and light conditions. It is probable, however, especially on bright days, that temperature differences of as much as 1° C. might have occurred between the different jars in their experiments, so that the authors were actually measuring a temperature effect rather than a direct light effect. In view of the writer's (5) own experience in growing plants under different intensities of light it seems improbable that the light from a window during winter, especially in Leningrad, would be too intense for maximum plant growth.

Light, by increasing soil and air temperatures, does, however, produce a pronounced acceleration in germination, early growth of the seedling, and rate of exhaustion of stored materials. This indirect effect of light on seedling activity is of considerable ecological importance and probably explains in part why seeds of many species of plants fail to produce as vigorous seedlings in the shade of a dense forest cover as they produce in the open, especially in northern latitudes. In the case of young conifers the rate of utilization of the endosperm may be so retarded by low temperatures that the seed is sloughed off before the food reserve has been exhausted. The weak, slender appearance of white pine and white spruce seedlings, which the writer has observed in those that germinated in the shade of an aspen stand compared to the appearance of those germinated in the open, indicates that full use of the reserve food in the seed may not always occur in the shade.

Summary

1. Maize seedlings were grown in the light and in the dark to determine if light, aside from its photosynthetic effect, had an effect upon the rate of utilization of the stored foods in the grain and the resultant growth of the seedlings.

2. When temperature conditions were carefully controlled there appeared to be no difference between the weights of residual seeds whether the seedling grew in the dark or in the light.

3. During the first four or five days after germination seedlings in the dark increased in dry weight just as rapidly as seedlings in the light. Thereafter, those in the light accumulated dry matter much faster, but this change is believed to be due to photosynthetic activity in the light.

4. An increase in temperature from 21.7° to 23.0° C. caused an increase in the dry weight of maize seedlings of 10 percent in spite of the fact that the plants at the lower temperature were in the light and the others in the dark.

5. The effect of light on germination and initial growth through its action in increasing soil temperatures is undoubtedly of considerable ecological importance in determining seedling establishment, especially in forested regions of northern latitudes.

The writer wishes to acknowledge the kindness of Professor R. B. Harvey in providing space in the greenhouse and constant temperature rooms of the Department of Agricultural Botany, University of Minnesota, for the first two experiments here described.

LAKE STATES FOREST EXPERIMENT STATION,
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STUDIES IN ONAGRACEAE VII. THE SUBGENUS PACHYLOPHIS OF THE GENUS *OENOTHERA*

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(Received for publication May 11, 1931)

As here presented, Pachylophis is made to be more inclusive than is customary, and its definition has to be somewhat changed. The reasons for including *Oenothera primiveris*, *Oe. xylocarpa*, and *Oe. tubifera* in the same subgenus with *Oe. caespitosa* will be presented in the discussion under these species.

For this paper I have had the privilege of working in the Gray Herbarium (G) and of borrowing material from the United States National Herbarium (US). I shall cite also material from the Pomona College Herbarium (P). To Dr. B. L. Robinson and to Dr. W. R. Maxon I am therefore greatly indebted, and to Dr. F. W. Pennell of the Philadelphia Academy for photographs of Nuttall specimens.

PACHYLOPHIS

Subgenus **Pachylophis** (Spach) Jepson, Man. Fl. Pls. Calif., 680. 1925.

Pachylophis, as genus, Spach, Hist. Veg. 4: 365. 1835; Raimann, in Engler & Prantl, Die Nat. Pflanzenfam. 3, Abt. 7: 215. 1893. As sect., Endlicher, Gen. Pl., 1190. 1840.

Perennial, sometimes annual, chiefly acaulescent, caespitose herbs. Leaves subentire or pinnatifid. Flowers vespertine, white or yellow, becoming reddish. Stamens subequal. Stigma with 4 linear lobes. Capsule cylindric to lance-ovoid, tapering gradually toward the tip, not winged, usually ridged, often tuberculate, thick-walled. Seeds in 2 rows in each cell, bordered along each side, these borders usually drawn together so as to made a deep furrow along the raphe.

Type species, *Oe. caespitosa* Nutt.

KEY TO SPECIES

Capsule not beaked, somewhat 4-angled, not winged nor tuberculate; flowers apparently yellow; plants caulescent with linear to lanceolate, almost entire leaves. Central Mexico ..... 4. *Oe. tubifera.*

Capsule beaked. Mostly in United States.

Flowers white, aging pink ..... 1. *Oe. caespitosa.*

Flowers yellow, aging red.

Capsule 3.5–6 cm. long; plant with dense short soft pubescence; leaves with large terminal lobe and few small lateral ones. Pine belt, Tulare and Mono Counties, Calif.; Washoe Co., Nev. .... 2. *Oe. xylocarpa.*

Capsule 2–3.5 cm. long; plant villous or pilose; leaves rather deeply and regularly pinnatifid. Deserts, S. Calif. to Utah and Texas ..... 3. *Oe. primiveris.*

1. OENOTHERA CAESPITOSA Nutt., in Fraser Cat., n. 53. 1813; Sims, Bot. Mag. 39: pl. 1593. 1813.

Oe. caespitosa of Leveille, Mon. Onoth., 67, 1902, in large part. *Pachylophis caespitosa* (Nutt.) Raimann, Engl. & Prantl, Die Nat. Pflanzenfam. III, 7: 215. 1893; Small, Bull. Torrey Club 23: 181. 1896.

Caespitose perennial from a rather thick taproot, usually acaulescent or nearly so, sometimes caulescent and 1–3 dm. high, glabrous, or glabrate with ciliate margins and veins, or densely and finely canescent, or even villous; leaves oblanceolate, the blades 3–12 cm. long, 1–2 cm. wide, subentire to sinuate-dentate or pinnatifid, on winged petioles of about the same length; flowers fragrant, opening in the evening; calyx-tube 5–8 (20) cm. long, often tinged with red, glabrous within; calyx-lobes 2.5–3.5 cm. long, with scarcely any free tips; petals white, aging pink, broadly obcordate, 2.5–4 (5) cm. long; stamens subequal, glabrous, two-thirds as long as petals; anthers 10–14 mm. long, glabrous; pistil equal to petals; style glabrous; stigma-lobes 5–8 mm. long; capsule coriaceous, lance-ovoid, 1–3 (3.5) cm. long, attenuate into a stout beak, quite sessile, with or without low rounded tubercles on the angles; seeds dark brown, ca. 3 mm. long, obovoid, minutely cellular-roughened, conspicuously furrowed along the raphe.

KEY TO VARIETIES

The capsule sessile and quite ovoid, often curved and asymmetrical, spreading.
- Capsule distinctly tubercled.
  - Leaves subentire to sinuate-dentate. United States.
    - Plant acaulescent.
      - Herbage glabrous or nearly so. Dakota to Wyo.... 1*a*. var. *typica*.
      - Herbage pubescent.
        - Plant canescent with a fine appressed pubescence. E. Wash. to Mont. and Nevada..... 1*b*. var. *purpurea*.
        - Plant with spreading hairs.
          - Hair soft, somewhat curly; leaf-blades 1.5–3 cm. wide, 3–6 cm. long. E. Utah..... 1*c*. var. *Jonesii*.
          - Hair stiff; leaf-blades 5–8 mm. wide, 1–2 cm. long. E. and S. Nevada and adjacent Utah. 1*d*. var. *crinita*.
    - Plant caulescent, 1–3 dm. tall, with spreading hairs on leaf-margins, veins, and calyx-tube. E. Colo. and New Mex..... 1*e*. var. *eximia*.
  - Leaves deeply divided into narrow lobes, sparingly villous. Lower California..... 1*f*. var. *Brandegeei*.
- Capsule not tubercled.
  - Capsule glabrous or somewhat villous.
    - Plant commonly conspicuously villous on leaf-margins and veins. Utah to Ore. and Mont..... 1*g*. var. *montana*.
    - Plant glabrous. St. Anthony, Idaho..... 1*h*. var. *psammophila*.
  - Capsule and foliage appressed-canescent. S. New Mexico and Arizona..... 1*i*. var. *australis*.

The capsule pedicelled, straight, symmetrical, erect, cylindric-ovoid.
- Plant villous-hirsute. Calif. to Utah, Ida., Ore..... 1*j*. var. *marginata*.
- Plant almost glabrous. Inyo Co., Calif. and adjacent Nevada. 1*k*. var. *longiflora*.

1*a*. **Oenothera caespitosa** Nutt. var. **typica** n. nom.

Oe. caespitosa Nutt., l. c. *Oe. scapigera* Pursh, Fl. N. Am. 1: 263. 1814. *Pachylophis glaber* (pub. *glabra*) A. Nels., Bull. Torrey Club 31: 242. 1904. *P. Nuttalliana* Spach, Ann. Mus. Par. ser. 3, 4: 356. 1835. *P. Nuttallii* Spach, Hist. Veg. Phan. 4: 365. 1835.

Glabrous or nearly so, sometimes more or less pubescent on margins of leaves and on veins; calyx-tube 5–8 cm. long; capsule 1–2 cm. long, tubercled on angles.

Type locality, "on the banks of the Missouri, from White river to the Mandans." Ranging from the Dakotas to Montana and Wyoming and possibly eastern Oregon. Representative material, type coll., *Nuttall*, photo of Philadelphia material; NORTH DAKOTA: Upper Missouri, *Coens in 1874* (G); Glen Ullen, *Holzinger 8* (US). SOUTH DAKOTA: Hot Springs, *Bates in 1896* (G), *Rydberg 707* (G, US); Ft. Pierre, *E. T. & S. A. Harper in 1891* (P). CANADA: Lethbridge, Assiniboia, *Macoun 4949* (G), *10618* (US). NEBRASKA: Scott's Bluff, *Rydberg 114* (G). MONTANA: Gardiner, *Blankinship in 1899* (P); Midvale, *Umbach 42* (US); Glacier Park Station, *Standley 17719* (US). WYOMING: Gros Ventre Basin, *Nelson 926* (G); Yellowstone Park, *Knowlton in 1888* (US). OREGON: Crooks River, *Cusick 2633* apparently this (G, P, US).

There is considerable variation in the leaf-margin and in the pubescence. The Bates collection from Hot Springs has the leaves almost entire instead of sinuate-dentate. *Cusick 2633*, *Rydberg 114* and *Holsinger 8* are quite villous on the hypanthium and leaf-margins.

1*b*. **Oenothera caespitosa** Nutt. var. **purpurea** (Wats.) n. comb.

Oe. marginata var. *purpurea* Wats., Bot. King Exped., 108. 1871. *Pachylophis canescens* Piper, Contr. U. S. Nat. Herb. 11: 409. 1906.

Densely canescent throughout with a fine appressed pubescence; capsule 1–2 cm. long, with low rounded tubercles on angles.

Type locality, East Humboldt Mts., Nevada. Ranging along the eastern parts of Washington and Oregon to Montana, Nevada, Wyoming. Representative material, WASHINGTON: Sentinel Bluffs, *Cotton 1345*, type coll. *canescens* (G, US). OREGON: Narrows, Harney Co., *Peck 5843* (G); Harper Ranch, Mathew Co., *Leiberg 2103* (G, US). IDAHO: Challis, Custer Co., *Macbride & Payson 3230* (G, P, US); Pocatello, *Palmer 37*, in 1893 (US); Martin, Blaine Co., *Macbride & Payson 3042* (US). NEVADA: East Humboldt Mts., *Watson 412*, type (G); Elko, *A. E. Hitchcock 924* (US). MONTANA: Shinbergers Ranch, S. W. Mont., *Watson 149* (G). WYOMING: Mammoth Hot Springs, *Mearns 823* (US), approaching var. *montana*, in pubescence.

With its appressed short pubescence this variety is a fairly definite entity, although material is often quite intermediate between this and the longer-haired *montana* which is relatively glabrous on the actual leaf-surfaces and which does not have tubercled fruits.

1c. **Oenothera caespitosa** Nutt. var. **Jonesii** n. var.

Leaves and habit of *montana*, but densely villous or even almost tomentose with short white, somewhat curly spreading hair; capsule ovoid, somewhat tuberculate. (Folia villosa aut subtomentosa; capsulis ovoideis, tuberculatis).

Type, Fish Springs, Grand County, Utah, *M. E. Jones, June 4, 1981*, Pomona College Herbarium No. 38813. Apparently a local variant in central eastern Utah, characterized by its rhomboid-ovate, subentire leaves, dense curly-villous condition, and tubercled ovoid capsules. Other material seen, UTAH: Thompsons Spring, *Jones in 1891* (P); Green River, *Jones in 1890* (P); *Tidestrom 1056* (US).

1d. **Oenothera caespitosa** Nutt. var. **crinita** (Rydb.) Munz, n. comb.

Pachylophus crinitus Rydb., Fl. Rocky Mts. & adj. plains, 598 & 1064. 1917.

Caespitose from a woody caudex; stems 3–8 cm. high; leaves densely hirsute or even pilose, crowded, the blades lanceolate, obtuse, sinuate, 10–15 mm. long, 3–5 mm. wide; calyx-tube 2–4 cm. long, hirsute; capsules 10–14 mm. long, 3–5 mm. thick.

Type locality, Rabbit Valley, Utah. Rather local in Utah and Nevada. Material seen, UTAH: Richfield, *Ward 167* (US); Rabbit Valley, *Ward 526*, in part, type (US). NEVADA: Charleston Mts., *Jaeger in 1925, in 1926, in 1927* (P); Ely, *Jones in 1907* (P); Currant, Nye Co., *Bentley 10* (US). This plant grows at rather high altitudes and is characterized by its small leaves, stiff pubescence and compact habit; plants from Charleston Mts. have stiffer pubescence than do the Utah ones. Some collections, such as Toyabe Forest, *Hitchcock 852* (US), resemble this in the pubescence but have larger leaves like those of *marginata*.

1e. **Oenothera caespitosa** Nutt. var. **eximia** (Gray) Munz, n. comb.

Oe. eximia Gray, Pl. Fendler., 45. 1849. *Pachylophus eximius* Woot. & Standl., Contr. U. S. Nat. Herb. **16**: 157. 1913. *P. exiguus*, by error, Rydberg, Bull. Torrey Club **30**: 260. 1903.

Caulescent, with stout stems 1–3 dm. tall; leaves pilose, especially on margins and veins; calyx-tube 10–15 cm. long, pilose; petals 2.5–4 cm. long; capsules ascending, rarely maturing, when mature they are 2.5–4 cm. long, with unusually large tubercles.

Type locality, Santa Fe Creek, New Mexico. Apparently a rare plant, of which I have seen the following material, COLORADO: Rocky Ford, *Crandall 242* (G); Canyon City, *Brandegee in 1873* (G); Pueblo, *Farwell 1051* (G), *Bodin 163* (US); Colorado Springs, *Jones 956* (P). NEW MEXICO: Santa Fe Creek, *Fendler 228*, type (G); mouth of Holy Ghost Creek, *Standley in 1908* (US). At first I thought this plant to be quite distinct from *Oe. caespitosa*, but it has the capsule of the same general shape, although more tubercled and ascending. It is like var. *montana* in having hair on leaf-

margins and veins, and like var. *marginata* in its caulescence and spreading hairs.

1*f*. **Oenothera caespitosa** Nutt. var. **Brandegeei** n. var.

Acaulescent, scattering-villous throughout; leaves with subovate terminal portion and pinnately dissected below that into narrow lanceolate lobes which point forward; calyx-tube 3–4 cm. long; petals ca. 1 cm. long; capsule 1–2 cm. long, lance-ovoid, well tubercled. (Planta acaulescens, villosa; foliis pinnatifidis, cum lobis linearo-lanceolatis; tubo calycis 3–4 cm. longo; petalis 1 cm. longis; capsulis 1–2 cm. longis, lanco-ovoideis, tuberculatis).

Type, from stony ridges, near Los Angeles Bay, Gulf of California, Lower California, *E. Palmer 542*, Dec., 1887, Gray Herbarium. Another sheet, *Palmer 582* (US) from Los Angeles Bay. This is the plant referred to by Brandegee (Proc. Calif. Acad., ser. 2, **2**: 157. 1889) as "Oenothera caespitosa Nutt. var. Leaves finely divided and villous.—El Campo Aleman."

1*g*. OENOTHERA CAESPITOSA var. MONTANA (Nutt.) Durand, Bot. Basin of Great Salt Lake of Utah, 164. 1859.

Oe. montana Nutt. in T. & G., Fl. N. Am. **1**: 500. 1840; *Pachylophis montanus* A. Nels., Bull. Torrey Club **26**: 128. 1899. *P. macroglottis* Rydb., l. c. **30**: 259. 1903. *P. hirsutus* Rydb., l. c. **31**: 571. 1904, in part. *P. caulescens* Rydb., l. c., in part.

Acaulescent; leaves canescent-pubescent on margins and often on veins of lower surface, otherwise glabrous or nearly so; calyx-tube 3–8 cm. long, somewhat villous; capsule ca. 2 cm. long, with sinuate ridges and occasional cross-veins, but no tubercles.

Type locality, "Plains of the Platte in the Rocky Mountains." Ranging from Montana and Oregon to New Mexico and Arizona. Representative material, MONTANA: Lima, *Jones in 1908* (P); Sedan, Gallatin Co., *B. J. Jones in 1901* (G). IDAHO: Soda Springs, *Mulford in 1892* (G); Salmon, Lemhi Co., *E. B. & L. B. Payson 1834* (G). OREGON: Muddy, John Day Valley, *Howell 402* (G). WYOMING: Mammoth Hot Springs, *Mearns 847* (US); Big Piney, Sublette Co., *E. B. & L. B. Payson 4358* (G); Golden Gate, Yellowstone, *A. & E. Nelson 5530* (G, P, US); Green River, *Jones in 1896* (P); Laramie Hills, *Nelson 6952* (G, P, US). COLORADO: Golden, *Jones 280* (P); Mancos, *Baker, Earle & Tracy 141* (G, P, US); Arboles, *Baker 496* (G, P, US); Norwood Hill, San Miguel Co., *Walker 485* (G, US). NEW MEXICO: Potato Canyon, Upper Canadian, *St. John 182* (G); Tierra Amarilla, Rio Arribe Co., *Eggleston 6590* (US). ARIZONA: Navajo Ind. Reservation, Carrizo Mts., *Standley in 1911* (G). NEVADA: Winnemucca, *Wooton in 1917* (US); mts. on Humboldt River, *Beckwith* (G). UTAH: La Sal Mts., *Jones in 1914* (P); American Fork Canyon, *Goodding 1172* (G, US); Alta, *Jones in 1910* (P). This variety is the prevailing one in the central Rocky Mountains. It is quite a variable thing; some plants are almost

glabrous and suggest var. *typica* but are not tubercled on the capsules, for example, Midway, Wasatch Co., Utah, *Carlton & Garrett 6683* (US). Others are glabrate, but somewhat tubercled, yet occur far south of the range of *typica*, as Dulce, New Mexico, *Standley 8176* (US). In general the tendency toward the southern part of the range is to have longer, more abundant hair and to approach in general appearance the var. *marginata*, for example, Grand Junction, Colo., *Jones in 1883* (P).

1*h*. **Oenothera caespitosa** var. **psammophila** (Nels. & Macbr.) n. comb.

Pachylophus psammophilus Nels. & Macbr., Bot. Gaz. **61**: 32. 1916.

Glabrous, caulescent, 1 dm. or more high; leaves entire or repand; capsules 3–3.5 cm. long, not tubercled.

Type locality, "sand dunes in the vicinity of St. Anthony, Idaho." Material seen, IDAHO: sand hills, 10 miles west of St. Anthony, *Merrill & Wilcox 888* (G, US), *Merrill 438* (US); sand hills near Egin, *Quayle 29* (P). This plant is glabrous like *typica*, but is caulescent and lacks tubercles on the capsules.

1*i*. **Oenothera caespitosa** var. **australis** (Woot. & Standl.) Munz, n. comb.

Pachylophus australis Woot. & Standl., Contr. U. S. Nat. Herb. **16**: 156. 1913.

Acaulescent or nearly so, finely cinereous throughout; leaves 1–2 dm. long, sinuate-pinnatifid; calyx-tube 10–18 cm. long; petals 4–5 cm. long; mature capsules not seen, young ones are appressed pubescent, and apparently not tubercled.

Type locality, South Fork of Tularosa Creek, Otero Co., New Mexico. Material seen, NEW MEXICO: Kingston, Sierra Co., *Metcalfe 1097* (G); Tularosa Creek, *Wooton in 1899* (US). ARIZONA: Montezuma Canyon, Huachuca Mts., *Goodding 382* (G); Lowell, *Carlson in 1915* (US). In its fine close pubescence this variety resembles the more northern *purpurea*, but lacks the tubercles of the capsule of that plant.

1*j*. **Oenothera caespitosa** var. **marginata** (Nutt.) Munz, n. comb.

Oe. marginata Nutt., H. & A., Bot. Beechey, 342. 1838. *Pachylophus marginatus* Rydb., Bull. Torrey Club **33**: 146. 1906. *Oenothera idahoensis* Mulford, Bot. Gaz. **19**: 117. 1894. *Pachylophus hirsutus* Rydb., Bull. Torrey Club **31**: 571. 1904, in part. *P. cylindrocarpus* A. Nels., Bot. Gaz. **47**: 429. 1909.

Villous-hirsute throughout, but especially on the leaf-margins and veins; usually caulescent; leaves usually sinuate-pinnatifid; capsule pedicelled, 3–4 cm. long, linear-cylindric, not recurved, scarcely ridged, with low tubercles.

Type locality, "Rocky Mountains in Upper California, about lat. 42°," probably in Idaho. The var. *montana* and this one are by all odds the two most common ones in the species, *montana* in the Rocky Mts. and *marginata* in the Great Basin to the west. Representative material of *marginata*,

WASHINGTON: Grande Ronde River, *Cantwell 77* (US). OREGON: Snake River, *Cusick 2542* (G, P, US); Steins Mt., *Howell 408* (G). IDAHO: Curlew Gulch, type coll. *idahoensis*, *Mulford in 1892* (G); Murphy, Owyhee Co., *Macbride 1031* (G, P, US); Boise, *Macbride 265* (G, US); Picabo, Blaine Co., *Macbride & Payson 2988* (G, P, US); Lewiston, *Sandberg, Mac Dougal & Heller 185* (G, P, US). Rocky Mts., *Nuttall*, type coll. (G). UTAH: Marysvale, *Jones 5375* (P, US); Zion Park, *Craig 1409* (P); Silver City, Juab Co., *Jones in 1896* (P). COLORADO: Dix, *Baker, Earle & Tracy in 1898* (P); Minturne, Eagle Co., *Osterhout 2546* (P); Montrose, *Shear 4846* (US). NEW MEXICO, Tunitcha Mts., *Standley 7693* (US); Burro Mts., Grant Co., *Metcalfe 200* (G, P, US). ARIZONA: San Francisco Mts., *MacDougal in 1891* (US); El Tovar, *Jones in 1929* (P); Walnut Canyon, *MacDougal 341* (G, US); Oracle, *Jones in 1903* (P); Hackberry, *Jones in 1884* (P); Nogales, *Tidestrom 739* (US). NEVADA: E. Humboldt Mts., *Heller 9509* (US); Canyon of the Truckee, *K. Brandegee 2048* (G, US); Mt. Grant, Mineral Co., *Heller 10904* (G, US); Charleston Mts., *Jaeger in 1925* (P). CALIFORNIA: Panamint Mts., *Coville & Funston 535* (G, US); Bear Valley, San Bernardino Mts., *Munz 5678* (P); Providence Mts., *Munz, Johnston & Harwood 4032* (P, US).

In Utah particularly, *montana* and *marginata* intergrade freely. *Goodding 840* (G, US) from Diamond Valley; *Jones 1746*, in part (P, US) from Salt Lake City; *Jones in 1891* (P) from Dugway are examples with the capsules so weakly tubercled as to be quite intermediate.

1*k*. **Oenothera caespitosa** var. **longiflora** (Heller) Munz, n. comb.

Anogra longiflora Heller, Muhlenbergia 2: 224. 1906. *Pachylophus longiflorus* Heller, Muhlenbergia 4: 40. 1908.

Plant glabrate except for few possible hairs about the margins of leaves, ovaries, and calyx-lobes, which latter may also be finely glandular-puberulent; otherwise much as in *marginata*.

Type locality, Silver Canyon, White Mts., east of Laws, Inyo Co., California. Material seen, Silver Canyon, *Heller 8219*, type collection, (G, US). NEVADA: Miller Mt., Candelaria, *Shockley 332* (G). This is rather a minor entity, for which there is not yet sufficient material known. The Heller collection is quite glabrous and distinct, but the Shockley one has a few hairs; both are finely glandular.

2. OENOTHERA XYLOCARPA Coville, Contr. U. S. Nat. Herb. 4: 105, pl. 8. 1893.

Anogra xylocarpa Small, Bull. Torrey Club 23: 174. 1896.

Acaulescent perennial with thick vertical root which branches below; caudex short, surmounted by crown of leaves at surface of ground; leaf-blades pinnately parted, often spotted red, 2–7 cm. long, 1–3 cm. wide, broadly oblanceolate to obovate in outline, with a short dense soft sometimes canescent pubescence, terminal lobe much the largest, ovate to cordate to

suborbicular, coarsely and irregularly dentate or denticulate or subentire, obtuse or rounded, 1–5 cm. long, 1–3 cm. wide, lateral lobes usually small, few, lanceolate to oblong-ovate, 3–10 mm. long, 2–6 mm. wide, often irregularly coarsely toothed, sometimes quite lacking; petioles pubescent, about as long as blades; flowers vespertine; calyx-tube slender, almost villous-pubescent without, 2.5–4.5 cm. long, finely puberulent within the tubular portion; calyx-lobes separate and reflexed in anthesis, pubescent, lance-linear, 2–3 cm. long, 3–4.5 mm. wide, without free tips; petals bright yellow, aging salmon-red, cuneate-obcordate, 2.5–3 cm. long, slightly wider, with broad terminal sinus 4–5 mm. deep; stamens pale yellow, equal, ca. two-thirds the length of the petals; filaments 13–15 mm. long, dilated toward the base; anthers glabrous, 9–11 mm. long; style pale yellow, extending to base of sinus of petal, glabrous; stigma-lobes 4–5 mm. long; capsule somewhat woody, 3.5–6 cm. long, the capsule proper 7–8 mm. thick at base, tapering gradually to a long slender apex, square in cross section, the lower half winged at the angled sutures, each wing 2–3 mm. long and gradually narrowed up the capsule, a broad median thickened rib down the middle of each face of the capsule; seeds in 2 rows in each cell, brownish, obovoid, 2–2.5 mm. long, narrowly obovoid, truncate at summit, angled, roughened and minutely tubercled, with a broad flat non tubercled raphe.

Type locality, west side of Whitney Meadows, later called Volcano Meadows, in the upper Kern River Basin, Tulare Co., California. Material seen, CALIFORNIA: Fish Creek, S. E. Tulare Co., *Hall & Babcock 5214* (US); trail to Mt. Whitney, *Culbertson 4526* (G, P); north side of Mt. Whitney, *Dudley 2490* (Stanford); Volcano Meadows, *Coville & Funston 1627*, type coll. (G. Stanford), *Hall & Babcock 5489* (Stanford); Tunnel Ranger Station, *Ferris 3753* (Stanford); 35 miles north of Bishop, in Mono Co., *Munz 11084* (P). NEVADA: Galena Creek, Washoe Co., *Heller 10225* (Stanford, US); Mt. Rose, *Heller 9894* (Gray, Stanford).

This species was originally ascribed to the "subgenus Euoenothera" by Coville, later transferred to Anogra by Small, then to the subgenus Lavauxia by Jepson, Man. Fl. Pls. Calif., 682, 1925. It is not close to any other species and might well be placed in a new subgenus. Its long attenuate capsules, winged in the lower half (instead of the upper as in Lavauxia, or throughout as in Megapterium) and its seeds (neither terminally winged as in Lavauxia and Megapterium, nor with a narrow groove as in Pachylophis) might be taken as evidence for such recognition. On the other hand, its caespitose habit, the general shape of the capsules, even though slightly winged at the base, the absence of free tips to the calyx-lobes, the pubescence, all these indicate an affinity to Pachylophis. Moreover, the seeds are nearer that group than to Lavauxia or Megapterium. If the margins on the raphial face were drawn together to make a groove, the seeds would be quite typical of Pachylophis.

3. OENOTHERA PRIMIVERIS Gray, Pl. Wright. **2**: 58. 1853.

Lavauxia primiveris Small, Bull. Torrey Club **23**: 182. 1896. *Oenothera caespitosa* var. *primiveris* Leveille, Mon. Onoth., 71. 1902. *Oe. Johnsoni* Parry, Amer. Nat. **9**: 270. 1875. *Oe. caespitosa* race

Johnsoni Levl., l. c. 70. *Pachylophis Johnsoni* Rydb., Fl. Rocky Mts., 598, 1064. 1917. *Oenothera bufonis* M. E. Jones, Contr. West Bot. **8**: 28. 1898. *Lavauxia lobata* A. Nels., Bot. Gaz. **47**: 429. 1909.

Apparently annual or winter annual, with long tap root system, caespitose, acaulescent or nearly so, occasionally with stems up to 1 dm. long; villous or pilose-pubescent throughout, the leaf-surfaces sometimes glabrate; leaf-blades oblanceolate in outline, 1–12 cm. long, 0.3–3 cm. wide, occasionally subentire, commonly deeply and rather regularly deeply pinnatifid into lanceolate to ovate lobes which are in turn toothed or lobed, all lobes usually quite rounded; leaf-blades gradually narrowed into somewhat winged petioles of less length, expanded at very base; calyx-tube slender, 2–6 cm. long, villous without, minutely puberulent within the lower half; calyx-lobes ordinarily reflexed in pairs at anthesis, lance-linear, 15–28 mm. long, 3–5 mm. wide, without free tips, frequently tinged reddish; petals canary yellow, aging orange-red, cuneate-obovate, 2–3 cm. long (in depauperate plants, sometimes 8–12 mm.) and almost as wide, with sinus 4–5 mm. deep; stamens subequal, filaments ca. two-thirds length of petals, slightly flattened especially toward base, anthers glabrous, 8–10 mm. long; style about equaling petals, glabrous, ordinarily 6–8 mm. long; capsule pilose, square in cross-section, with heavy rib down middle of each face, reticulate, not winged nor tuberculate, gradually tapering to attenuate apex, 6–8 mm. thick at base, 2–3.5 cm. long; seeds in 2 rows in each cell, brown, somewhat roughened-tuberculate, 2.5–3 mm. long, with narrow raphal groove.

Type locality, dry hills near El Paso, Texas. Extending widely over the deserts from California and Nevada to Utah, Texas, and Sonora. Representative material, CALIFORNIA: Panamint Mts., *Coville & Funston 753* (US); Darwin Mesa, Argus Mts., *Jones in 1897*, type of *bufonis* (P); Mojave, *Munz 10067* (P); Barstow, *Jones in 1924* (P); Ludlow, *Munz & Harwood 3420* (P, US); Shavers Well, Colorado Desert, *Munz & Keck 4748* (P); Banning, *Spencer 1819* (G). LOWER CALIFORNIA: Calmalli, *Purpus 224* (US); San Quentin Bay, *Palmer 663* (US). SONORA: Sonoyta, *Mearns 2731* (US); Hermosillo, *Rose, Standley & Russell 12407* (US). ARIZONA: Wellton, *Harrison 3624* (US); Tucson, *Rose 11892* (US); Kingman, *Jones in 1903* (P); Duncan, *Durant in 1920* (P). NEVADA: Goldfield, *Jones in 1907* (P); Good Springs, *Jones in 1905* (P); Meadow Valley Wash, *Goodding 2172* type coll. of *lobata* (G). UTAH: St. George, *Parry 64*, type coll. *Johnsoni* (G), *Jones in 1880* (P). NEW MEXICO: Organ Mts., *Wooton in 1900* (US); Mesilla Valley, *Wooton in 1905* (P, US); near El Paso, *Wright 1376*, type (G). TEXAS: El Paso, *Jones in 1884* (P); Rio Grande Valley, *Mex. Bound Survey* (US).

Tentatively referred to Pachylophis by Gray in the original description, it is to my mind to be referred there now on the basis of caespitose habit, wingless elongate capsule, and especially the narrow furrowed seeds. Gray's description was based on a collection by C. Wright whose plants were quite depauperate, with leaves only 2–5 cm. long and petals 8–12 mm. long. It is probably because of this fact that Parry, Jones and Nelson have described the large more common plants as new species. I have seen type material

of all three of their species and am perfectly satisfied that they are all synonyms of a species with wide range, comparable to that of *Oenothera chamaenerioides* and *Oe. refracta*. There is of course variation in the length of capsules, lobing and pubescence of leaves, size of flowers, caulescence, etc., but I have seen nothing to which I would give even varietal rank.

4. OENOTHERA TUBIFERA Seringe, ex D. C., Prodr. 3: 50. 1828.

Lavauxia tubifera Rose, Contr. U. S. Nat. Herb. 8: 329. 1905.

Perennial from simple or few-branched caudex with fusiform fleshy root which may be somewhat branched; subacaulescent or with few arching stems with the tips turning down, winged, 5–15 cm. long, greenish but strigose-pubescent throughout; leaves in tuft at base and also scattered along stems; radical ones linear to lanceolate, acute to acuminate, gradually narrowed at base into winged petiole 1–3 cm. long which becomes widest nearest insertion on caudex; cauline leaves about half as long, more nearly sessile and all tending to be erect; calyx-tube 8–10 cm. long, strigose without, glabrous within; calyx-lobes separate and reflexed in anthesis, somewhat reddish, linear-lanceolate, 20 mm. long, 3 mm. wide, without free tips; petals apparently yellow, drying reddish, broadly obovate, ca. 2 cm. long, equally wide with terminal sinus 2–3 mm. deep; stamens subequal, filaments ca. 10 mm. long, very slightly flattened, anthers glabrous, linear, 7–8 mm. long; style at least in some specimens projecting about half the length of the stamens, with stigmatic lobes ca. 5 mm. long and divaricate; capsule 4-angled but not at all or scarcely winged, 2–3 cm. long, gradually narrowed toward summit, not beaked, not woody, dehiscing along angles and with thick prominent vein down middle of each face; seeds brown, in 2 rows in each cell, somewhat mottled, fairly smooth and shining, somewhat ovoid-cylindrical, with narrow furrow along the raphe, 3 mm. long, 2 mm. thick.

Type locality, Mexico; the description being based on pl. 377 of Mocino and Sesse, Icon. Ined. Through the kindness of Dr. I. M. Johnston I have a tracing of the tracing at Gray Herbarium and I agree with Rose, l. c., that the plants he collected agree perfectly with this plate. Rose gives no reason for placing the species in Lavauxia. Its fruits do suggest some of the wingless forms of *Oenothera taraxacoides*, but its seeds are decidedly those of Pachylophis. Material seen, MEXICO, HIDALGO: between Somoriel and Las Lojas, *Rose, Painter & Rose 9240* (US); Sierra de Pachuca, *Rose & Hay 5622* (US). FEDERAL DISTRICT: Cima, *Pringle 10507, Aug. 30, 1906* (G), *Aug. 26, 1906* (US), *13501, Aug. 30, 1905* (US).

ADDITIONS TO AND CORRECTIONS OF PREVIOUS PAPERS

1. **Oenothera kernensis** n. sp.

Annual, erect, with few spreading branches from base, 8–12 cm. tall, somewhat canescent throughout, minutely glandular-pubescent in inflorescence; leaves well distributed on the plant, basal leaves oblanceolate, 10–15 mm. long, 3–5 mm. wide, almost sessile, obtuse or acute, thickened at very tip, subentire to denticulate; cauline leaves somewhat reduced, but abundant, lance-linear, 5–20 mm. long, 1–2.5 mm. wide, denticulate, sessile or nearly so, those of lateral branches erect; flowers few, solitary in upper axils

in sort of a lax raceme; calyx-tube 2–3 mm. long, pubescent without and within; calyx-lobes lanceolate, reflexed separately in anthesis, 5–6 mm. long, without free tips in bud; petals bright yellow, with 2 small reddish spots near base, obovate, 1 cm. long, 8–9 mm. wide; longer stamens 4–5 mm. long: glabrous; shorter ones ca. 2 mm. long; anthers 1–1.5 mm. long; pistil slightly exceeding longer stamens, puberulent at very base; stigma globose, barely 1 mm. thick; capsule ascending, somewhat curved, cylindric-clavate, 2–2.5 cm. long, 1–1.5 mm. thick, not beaked, pubescent, gradually narrowed into a pedicel 4–6 mm. long; seeds narrowly obovoid, ca. 0.5 mm. long. (Annua, erecta, 1 dm. alta, canescens; foliis sessilibus aut subsessilibus, oblanceolatis aut linearo-lanceolatis, denticulatis, 5–20 mm. longis; floribus paucibus, luteis; hypanthio 2–3 mm. longo; lobis calycis lanceolatis, 5–6 mm. longis; petalis obovatis, 1 cm. longis; stigmato globoso; capsulis cylindrico-clavatis, 2–2.5 cm. longis, pedicellatis.)

Type, from east slope of Walker Pass, Freeman Canyon, Kern Co., California, at 4500 ft. elevation, *F. W. Peirson 8822*, May 13, 1930, Pomona College Herbarium No. 170957; isotype in Herbarium Peirson. This species, collected by Messrs. Peirson and J. T. Howell, is a surprising new plant. Mr. Peirson reports that it grew on sandy slopes and openings and was abundant for a distance of two or three miles. Undoubtedly more material will somewhat change the description as above given, particularly as to vegetative parts, but the plants Mr. Peirson brought in are sufficient to indicate thoroughly the general nature of the plant. The pedicelled capsules throw it into the subgenus Chylismia, although it is not near any of the previously known species, since the other annuals are not so leafy throughout and tend to have more numerous stems and branches. In flower size and appearance, it suggests *Oe. bistorta* Nutt.; and in pubescence and manner of growth with its arching stems and secund leaves, it resembles *Oe. alyssoides* var. *villosa* Wats., both of which are Sphaerostigmas. But its clavate pediceled capsules place it in Chylismia.

2. OENOTHERA SCAPOIDEA T. & G. var. TORTILIS Jeps., Man. Fl. Pls. Calif., 687. 1925, of the subgenus Chylismia, was referred by me to possible synonymy under *Oe. clavaeformis* var. *typica* (Amer. Jour. Bot. 15: 236. 1928). Through the kindness of Dr. Jepson I have now had opportunity to examine the type and find that it should be in synonymy under *Oe. multijuga* var. *parviflora* (Wats.) Munz.

3. OENOTHERA FLAVA (A. Nels.) Garrett, Spring Flora of the Wasatch region, 4th edition, 1927. Mr. Garrett has kindly called my attention to this combination, which should replace mine as of date of 1930, Amer. Jour. Bot. 17: 361.

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MACROSPOROGENESIS AND THE DEVELOPMENT OF THE MACROGAMETOPHYTE OF *LYCOPERSICON ESCULENTUM*

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Very little information concerning macrosporogenesis and the development of the embryo sac in the Solanaceae is to be found in the literature, and little indeed concerning the tomato, *Lycopersicon esculentum* Mill. Hofmeister (1858) made a study of the mature embryo sacs of three Solanaceous plants, *Hyoscyamus orientalis*, *Scopolina atropoides*, and *Salpiglossis picta*, and gave a brief description of them. Jönsson (1881), however, was the first to make a study of the development of the embryo sac in this family. In *Saracha Jaltomato* he found that the macrospore mother cell forms four daughter cells. The inner cell develops into a normal eight-nucleate embryo sac. Cases in which the embryo sac is formed in this manner have been reported by Guignard (1882) for *Nicotiana tabacum* and *Cestrus splendens*, by Souéges (1907) for *Atropa belladonna*, by Palm (1922) for *Nicotiana tabacum* Deli-tabak, and by Svensson (1926) for *Hyoscyamus niger*.

Nanetti (1912) described a modified lily type of embryo sac development for *Solanum muricatum*, and Young (1923) found the lily type (?) of development in *S. tuberosum*.

Mrs. Lesley (1926) has described certain of the prophase stages of macrosporogenesis in tomato. So far as the writer is aware, no detailed study has been made of the stages between diakinesis and that of the fully mature embryo sac.

MATERIALS AND METHODS

For this investigation, material from two well-known varieties of cultivated tomatoes, Bonny Best and Greater Baltimore, was used. In 1925 and 1926 buds and mature flowers were collected from plants growing in the greenhouses of the Purdue Agricultural Experiment Station. The very young buds were placed in the fixing fluids intact. The tips of the older buds were cut away to aid penetration and, in the case of mature flowersf the pistil was dissected out and fixed. Formal-acetic-alcohol (6.5 cc. o, formaldehyde, 2.5 cc. of glacial acetic acid, and 100 cc. of 50 percent alcohol) and Schaffner's chromo-acetic solution were used as fixatives. Good preparations showing the formation of the macrospores and of the embryo sac were obtained from material in both fixatives. Both cross and longitudinal sections were cut 12 μ in thickness, mounted serially, and stained either in

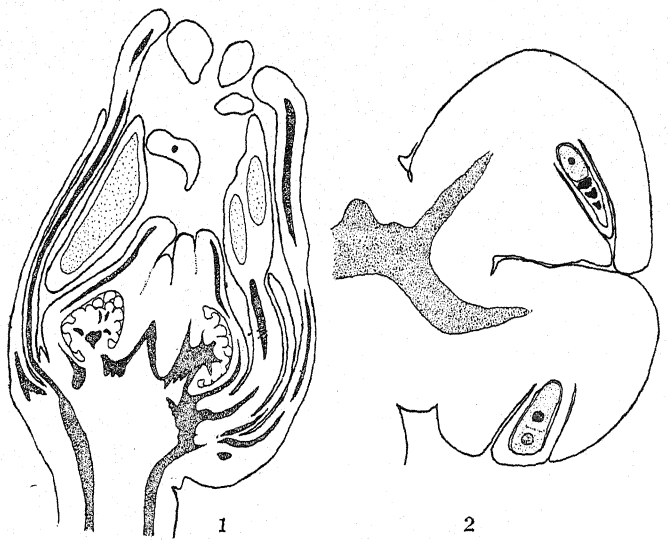
[1] Papers from the Department of Genetics, Agricultural Experiment Station, University of Wisconsin, No. 121. Published with the approval of the Director of the Station.

Heidenhain's iron-alum haematoxylin or in a combination of safranin and Delafield's haematoxylin.

No differences were found between the varieties studied in so far as concerns the development of the flower, the chromosome number, or the history of the macrospore mother cell and the embryo sac.

Development of the Ovule

As reported earlier by the writer (1927), the ovaries in the varieties studied consist of six united carpels with an enlarged placenta on the inner or axillary wall of each carpel on which the ovules are borne. This placenta is particularly evident in a longitudinal section through the ovary (text fig. 1). A similar massive placenta has been described by Young (1923) for



Text Fig. 1. Median longitudinal section through a flower bud, showing the ovules arising from large axial placenta.

Text Fig. 2. Median longitudinal section through two ovules showing the anatropous condition. The vascular strands end in the chalazal region.

potato and by Svensson (1926) in *Hyoscyamus niger*. The development of the ovule of the tomato conforms to the type found in most of the Sympetalae.

At the time when the microspore mother cells are in an early heterotypic prophase, the ovules appear as erect, rounded protuberances arising from the placental tissue. These protuberances soon become somewhat pointed, and growth is more rapid on one side of this mass of cells than on the other (Pl. XLVIII, fig. 3). As a result, each ovule becomes anatropous (text fig. 2). Chatin (1874) found anatropous ovules in *Nicotiana tabacum*, *N. rustica*, and *Datura Stramonium*. Young (1923) reports that the ovule of *Solanum*

tuberosum is not of the typical anatropous form since the embryo sac is considerably curved and thus suggests a transition toward the campylotropous form.

About the time when the single integument is beginning to develop, a hypodermal cell of the nucellus is differentiated as the archesporial cell. It is easily recognized by its somewhat greater size and by the fact that it stains more deeply than the surrounding cells (fig. 4). The nucleus contains prochromosome-like bodies, but these are not sufficiently numerous as to represent the chromosomes (fig. 11). The archesporial cell does not divide to form a primary parietal and a primary sporogenous cell, but functions directly as a macrospore mother cell. Mrs. Lesley (1926) occasionally found ovules in which two macrospore mother cells could be seen. During the course of this study no such cases were observed. As the macrospore mother cell enlarges it is surrounded at its apex and sides by a unicellular layer and its base is imbedded in the nucellus. When mature this macrospore mother cell is about twice as long as wide.

The integument begins as a meristematic outgrowth surrounding the ovule at a level just below that of the archesporial cell (fig. 4) and grows outward around the apical portion of the nucellus (figs. 5, 6). When the macrospore mother cell is in the heterotypic equatorial plate stage, this collar-like integument has reached the level of the apex of the ovule (fig. 7). Young microspores are to be found in the anther sacs at this stage of ovule-development. This is in agreement with the work of Mrs. Lesley (1926), who reports that the macrospore mother cells are in the prophase stages of the first reduction division when the pollen mother cells are in stages of cytokinesis. At the time when cell division is completed following the heterotypic division, the developing integument has almost surrounded the nucellus (fig. 8), and it has formed a micropyle of some length by the time the four macrospores are formed (fig. 9).

Cell division in the integument is in many planes, so that it grows in thickness as well as in length. When the macrogametophyte is mature, the thick integument has grown so far beyond it that the micropyle has a length of the combined diameters of 10–12 cells (fig. 10). A single trace of vascular tissue from the bundles of the placenta now extends through the funiculus and ends in the chalazal region of the ovule (text fig. 2).

Macrosporogenesis

At the onset of the heterotypic division the macrospore mother cell is about twice as long as wide; its nucleus is near the micropylar end of the cell and remains there throughout the heterotypic division. The chalazal end of the cell is more or less rounded, but is much narrower than the micropylar end (fig. 14).

The nucleus of the macrospore mother cell contains one conspicuous nucleolus somewhat imbedded in the chromatic network (figs. 12, 13).

In the early heterotypic prophases this chromatic network condenses (figs. 15, 16) until finally a dense synizetic knot is formed at one side of the nucleole (Pl. XLIX, fig. 17). On recovery from synizesis the spireme spreads out through the nuclear cavity (figs. 18, 19, 20) until the open spireme stage is reached. My results agree with those of Mrs. Lesley (1926), who found a true spireme and typical parasynapsis in the macrospore mother cells of the diploid tomatoes which she examined. At the open spireme stage there are evidences of a doubleness of the spireme (fig. 21). The chromosomes are formed by transverse segmentations of the spireme (fig. 22), and twelve pairs of chromosomes are clearly evident at diakinesis (figs. 23, 24). There seems to be no appreciable difference in size between the chromosome pairs at this stage. Mrs. Lesley (1926) also reports that the chromosomes of tomato are short rods showing no distinct individuality.

During the heterotypic prophases the macrospore mother cell has elongated so that, at the equatorial plate stage, it is about three times as long as wide (fig. 26).

As seen on the equatorial plate, the chromosomes may be divided into two classes according to size, namely, eight pairs of large chromosomes and four pairs of small chromosomes. In figure 25, a lateral view of an equatorial plate, and in figure 27, a polar view, twelve pairs can be counted. In a polar view of a mitotic division found in one of the cells of the integument, 24 chromosomes were counted (fig. 28).

After the heterotypic division, the macrospore mother cell is divided by a cell plate (fig. 29), which later splits (fig. 30), the micropylar cell being now about one-half the length of the chalazal cell. There is some growth in each cell before the homoeotypic division takes place. Figure 31 shows a late anaphase of this division. Twelve chromosomes can be counted in each of two of the chromosome groups. As a result of a second cell division a linear row of four macrospores is produced (fig. 32). The macrospore at the chalazal end of this row functions as the embryo-sac mother cell, the other three spores disintegrating. In no instances observed were less than four macrospores produced, and in all cases the chalazal spore became the functional embryo sac mother cell.

Chromosome Number

Winkler (1910) first reported the haploid number of chromosomes of tomato as 12. This number has been verified by East (1915), Winkler (1916), Lesley and Mann (1925), and Lesley (1926). These counts were made in pollen mother cells. In 1916 Winkler observed various polyploid numbers in tomato. Lesley (1925) reported a tomato plant having 36 chromosomes in its somatic tissues. Because of the behavior of the chromosomes during meiosis, this was considered to be a triploid form. In certain hybrids Lesley (1926, 1928) obtained forms having from 24 to 27 chromosomes in the somatic tissue. Lindstrom, 1929, reported the occurrence of

a hybrid tomato with 12 chromosomes. The chromosome counts in the cultivated varieties of tomato thus far examined are as follows:

Dwarf Aristocrat	2n* = 24	Lesley and Mann	1925
Dwarf Champion	2n = 24	Lesley	1926
	2n = 36	Lesley	1928
Globe	2n = 36	Lesley	1926
Balch's Fillbasket	n* = 12	Jörgensen and Crane	1927
	2n = 36	Jörgensen	1928
Early Dwarf Red	n = 12	Jörgensen and Crane	1927
Large Yellow	n = 12	Jörgensen and Crane	1927
Sutton's Best of All	n = 12	Jörgensen and Crane	1927
Livingston's Dwarf Aristocrat	2n = 36	Lesley	1928
Stone	2n = 36	Lesley	1928
Danish Export	2n = 36	Jörgensen	1928
	2n = 48	Jörgensen	1928

* n = gametic number; 2n = somatic number.

In the list of chromosomes found in angiosperms (Gaiser, 1930) the haploid number 12 is very common in the Solanaceae. Aside from the genus *Petunia* in which the haploid number is typically seven, there is a polyploid series in the family. Some exceptions, however, have been noted in certain hybrids and mutants (Blakeslee, Belling, and Farnham, 1923).

Development of the Embryo Sacs

During the rapid growth of the embryo sac mother cell, the apical and lateral nucellar cells become disorganized and ultimately disintegrate. These nucellar cells do not persist as do those of the potato, as shown in the figures of Young (1926). The nucleus of the functional macrospore does not divide until this cell has increased greatly in size (fig. 33) and in consequence of the disintegration of the nucellus its cell walls have come in contact with the cells of the integument. The first nuclear division takes place near the micropylar end of the macrospore (fig. 34), and one daughter nucleus moves toward the chalazal end of the embryo sac. Both nuclei then divide; in the meantime a large vacuole has appeared near the center of the cell (Pl. L, fig. 35). Figure 36 is a composite drawing from three sections of the same embryo sac showing three spindles and one nucleus in the spireme stage. The embryo sac appears short in this figure because of the inclination of its long axis to the plane of cutting.

After the third division the polar nuclei migrate toward the center of the embryo sac, leaving three nuclei at each end. A typical seven-celled embryo sac is formed (fig. 37). No figures were seen showing the method of cell division.

The egg apparatus at the micropylar end of the embryo sac consists of three more or less pear-shaped cells, the two synergids and the egg. In the early stages of development of the synergids, the nucleus is in the basal portion of each (fig. 37), and a vacuole lies above it toward the micropylar

end. With continued growth of the synergid, a large vacuole is formed near the base of the cell and the one near the micropyle disappears (fig. 41). During this time the nucleus changes its position, and later is found in the dense cytoplasm above the basal vacuole. The micropylar end of each synergid becomes more or less flattened, stains densely, and extends in a beak-like fashion into the micropyle. Coulter and Chamberlain (1903) point out that these beak-like projections of the synergids are common among the Sympetalae, a fact perhaps to be associated with the very heavy integument.

Each synergid develops a distinct filiform apparatus whose striations arise at or in the vicinity of the vacuole at the broad end of the cell and extend to the apex (fig. 41). The condition is similar to that described by Miss Brown (1917) for *Phaseolus vulgaris*. These striations appear to be made up of dense areas in the cytoplasm and of elongated vacuoles which extend from the large basal vacuole of the cell toward the apex. In the synergid shown in figure 41 certain of the elongated vacuoles appear to form canals that lead from the basal vacuole to the apical end of the synergid. A cross section of the micropylar end of a synergid at this stage shows that the denser regions are buried in the cytoplasm and are not accumulations of material in contact with the cell wall (figs. 39, 40). The many vacuoles in this region are clearly apparent in such a section.

The egg grows in such a way that its broad basal portion extends into the gametophyte beyond the synergids. When mature the egg is more or less pear-shaped (fig. 43), having a large vacuole in its apical region. The egg nucleus remains in the basal portion of the cell and is surrounded by dense cytoplasm. This nucleus is fully three times as large as that of a synergid.

The antipodal cells at the chalazal end of the embryo sac are more or less triangular in section and show a tendency to degenerate rather early. Guignard (1902) observed only vestiges of the antipodal cells in the mature embryo sac of *Datura laevis*, whereas in *Nicotiana tabacum* he found that the antipodals enlarge and persist for some time after the development of the endosperm is initiated. Young (1923), in potato, found that the antipodals were pressed against the chalaza by the growth of the embryo sac, later became flattened and finally disintegrated.

The polar nuclei come to lie close together near the egg; they fuse before fertilization. The nucleoles remain distinct after the fusion is completed (fig. 38). In *Datura laevis*, likewise, Guignard (1902) found the fusion of the polar nuclei to be completed before fertilization.

While the nucellar tissue is disintegrating the cells of the inner layer of the integument become elongated perpendicularly to the surface of the embryo sac (Pl. XLVIII, fig. 10). The cytoplasm of these innermost integumental cells becomes somewhat dense. This layer is sometimes called a "tapetum." No "tapetal" cells were found, however, having two nuclei, a condition which is typical of the tapetum of the anther sacs. Svensson

(1926) describes a similar nutritive layer about the embryo sac of *Hyoscyamus niger*. He finds starch kernels in this "tapetum." Young (1923) also illustrates a "tapetal" layer in potato. The tissue next outside this "tapetum" or nutritive layer shows evidences of disintegration at the time of fertilization.

In three instances remnants of the pollen tube were found in or near the micropyle. In one case the contents of the pollen tube was so dense that the male gamete nuclei could not be recognized with certainty (Pl. L, fig. 42). In each of the other cases the tip of the pollen tube lay between the synergids. One male gamete nucleus was closely appressed to the nucleus of the egg and the other male gamete nucleus was near the egg and at nearly the same level as the primary endosperm nucleus (fig. 43). Neither of the synergids had been broken by the entrance of the pollen tube.

Summary

1. With the appearance of the primordium of the integument, a single hypodermal cell of the nucellus is differentiated as the archesporial cell. This cell later functions as the macrospore mother cell.

2. In consequence of two divisions the macrospore mother cell produces a linear row of four macrospores.

3. The innermost (chalazal) macrospore functions as the embryo sac mother cell; the other three macrospores disintegrate.

4. A typical 8-nucleate embryo sac is formed. During its development the cells of the nucellus break down.

5. The inner layer of the integument, which is now in contact with the embryo sac, is differentiated as a nutritive layer.

6. The embryo sac is divided into seven cells, the three cells of the egg apparatus at the micropylar end, the three antipodals at the chalazal end, and a large binucleate primary endosperm cell.

7. The apices of the synergids are beak-like, extend into the micropyle, and each has a distinct filiform apparatus.

8. The polar nuclei come together near the egg and unite, so that the primary endosperm nucleus is found in close proximity to the egg.

9. The antipodal cells have disintegrated by the time of fertilization or shortly thereafter.

10. The pollen tube enters the ovule through the micropyle and passes between the synergids, neither of which is destroyed during the process of fertilization.

11. The haploid number of chromosomes in the Bonny Best and Greater Baltimore varieties of tomato is 12. The diploid number 24 was found in somatic divisions.

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EXPLANATION OF PLATES

All drawings were made with an Abbé camera lucida at table level. Spencer compensating oculars, a Spencer 8 mm. N.A. 0.60 apochromatic objective, and a Leitz oil-immersion N.A.1.32 objective were used. Figures 3–10 were drawn with a 10 × ocular and 8 mm. objective (× 360), figs. 11–31 and 38–40 with a 20 × ocular and the oil immersion objective (× 2900), figs. 32–37 and 43 with a 10 × ocular and the oil immersion (× 1550), and fig. 42 with a 5 × ocular and the 8 mm. objective (× 175). All the drawings have been reduced approximately one-third in reproduction.

PLATE XLVIII

FIG. 3. Young nucellus, showing hypodermal cell that is to become the archesporial cell.

FIG. 4. Young nucellus at a later stage, showing the origin of the integument.

FIG. 5. Young macrospore mother cell. Integument well defined.

FIG. 6. Ovule with macrospore mother cell shortly after synizesis.

FIG. 7. Ovule with thick integument. Macrospore mother cell in heterotypic division.

FIG. 8. Somewhat later than figure 7. Daughter cells formed by division of the macrospore mother cell.

FIG. 9. Ovule with linear row of four macrospores, three of which are disintegrating. The thick integument has surrounded the nucellus and formed a short micropyle.

FIG. 10. Ovule with an almost mature macrogametophyte. The integument has become 10 to 12 cell layers in thickness, the inner layer (the only one shown) forming a nutritive tissue. The nucellus is breaking down.

FIG. 11. Nucleus of archesporial cell showing prochromosome-like bodies.

FIGS. 12, 13. Nuclei of young macrospore mother cells. The chromatic material appears to be somewhat spread out along the linin network.

FIG. 14. Slightly older macrospore mother cell.

FIGS. 15, 16. The transition to synizesis.

PLATE XLIX

FIG. 17. Synizesis.

FIG. 18. Macrospore mother cell with nucleus recovering from synizesis. Cytoplasm is finely vacuolate.

FIGS. 19, 20. Spireme thread shortly after synizesis.

FIG. 21. Macrospore mother cell. Open spireme stage. In places the spireme is clearly double.

FIG. 22. Nucleus showing the formation of chromosomes.

FIGS. 23, 24. Diakinesis. Twelve pairs of chromosomes are present.

FIG. 25. Lateral view of a heterotypic equatorial plate showing 12 pairs of chromosomes.

FIG. 26. Macrospore mother cell with heterotypic equatorial plate.

FIG. 27. Polar view of heterotypic equatorial plate.

FIG. 28. Polar view of an equatorial plate in a somatic cell. 24 chromosomes are present.

FIG. 29. Cell-plate formation in a macrospore mother cell after the heterotypic division.

FIG. 30. Daughter cells formed by division of the macrospore mother cell. The chalazal cell is the larger.

FIG. 31. Daughter cells during the homoeotypic division. Late anaphase stage. Twelve chromosomes can be counted at one end of each spindle.

FIG. 32. Typical linear tetrad of macrospores, the three outer spores beginning to disintegrate.

FIG. 33. Functional macrospore beginning to enlarge.

FIG. 34. Binucleate embryo sac.

PLATE L

FIG. 35. Four-nucleate embryo sac.

FIG. 36. Division stages leading to the 8-nucleate stage. A composite drawing from three serial sections of the same embryo sac.

FIG. 37. Nearly mature embryo sac.

FIG. 38. Primary endosperm nucleus formed by the fusion of the two polar nuclei.

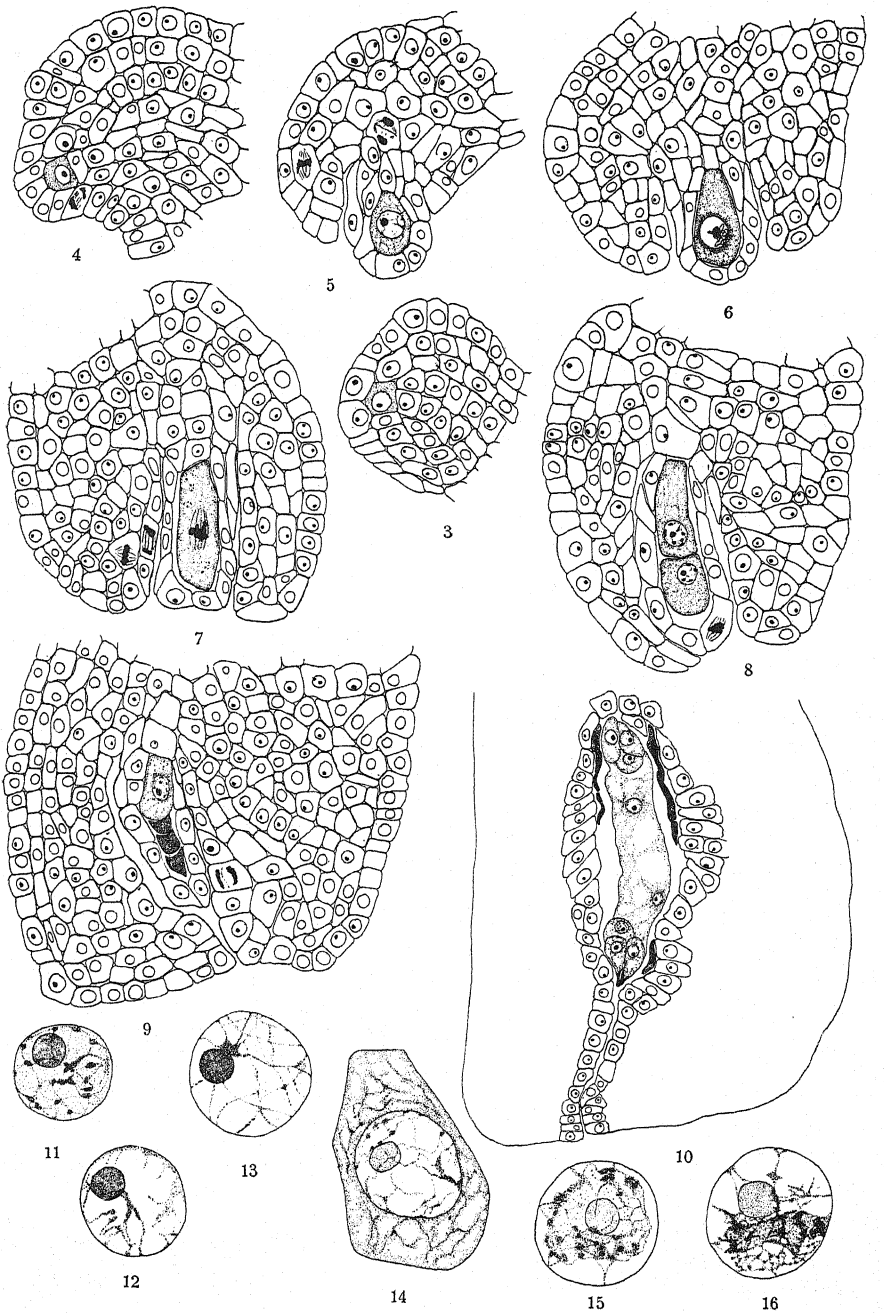
FIG. 39. Cross section of the synergids near the apex.

FIG. 40. Cross section of synergids near the nuclei.

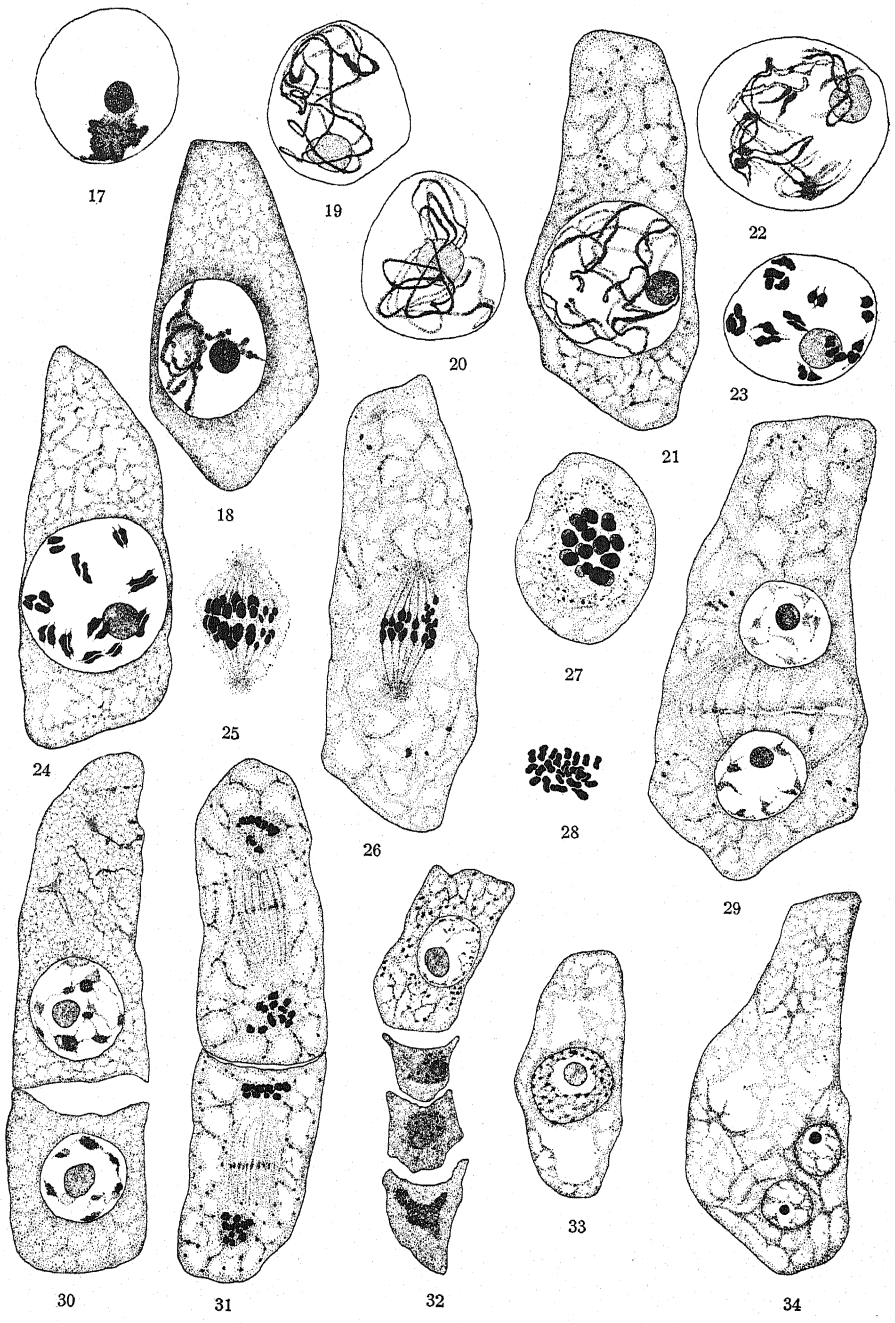
FIG. 41. Synergids with filiform apparatus.

FIG. 42. Portion of ovule showing pollen tube in the micropyle.

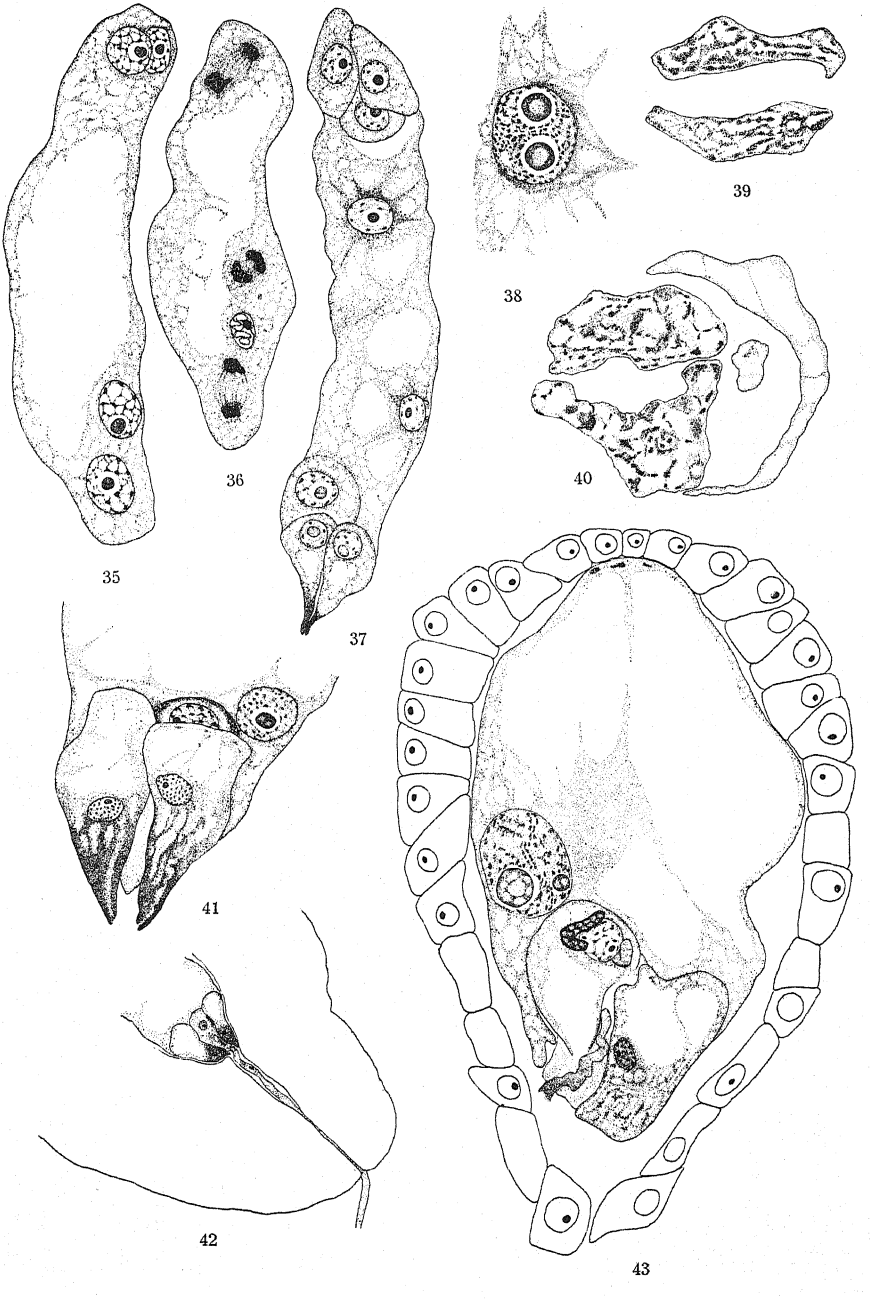
FIG. 43. A stage in fertilization. One male gamete nucleus is in close proximity to the egg nucleus. The other male gamete nucleus is above the egg nucleus and at nearly the same level as the primary endosperm nucleus. The tip of the pollen tube lies between the synergids and the egg. The three antipodals have almost disappeared. A composite drawing from three serial sections of the same embryo sac.



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POLLEN GRAINS IN THE IDENTIFICATION AND CLASSIFICATION OF PLANTS VI. POLYGONACEAE

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The pollen grains of the Polygonaceae are extremely various, apparently without any universal underlying similarities, so that it is useless to attempt to frame a definition for them. A study of the different forms, however, shows that they are related to each other and reveals several developmental tendencies which are remarkable and of far-reaching importance. In this family we see a suggestive illustration of how the ordinary tricolpate,[1] or three furrowed, type of grain with heavy walls and broad deep furrows might have given rise to the thin-walled type with furrows reduced to thread-like grooves, or entirely absent. Also we discover how the same tricolpate form of grain could have given rise to the many-pored, or cribellate, form of grain which reaches perhaps its highest development in the Chenopodiaceae and Amaranthaceae. As regards the morphology of their pollen grains the Polygonaceae are a transitional family, standing at the parting of the ways.

The basic form of the grains of this family can be typified by that of *Eriogonum* (Pl. LI, fig. 3). It is ellipsoidal, heavy walled with a thick exine of granular structure but perfectly smooth on its surface, and gashed almost from pole to pole by three long tapering furrows which function freely as harmomegathi,[2] closing up tightly when the grain dries and gaping open widely when it is moistened, with a scissor-like action at their pointed ends. In the middle of each furrow is a single roundish or elliptical germ pore, which is freely exposed when the furrow is open but more or less hidden when it is closed. This form of grain is always associated with insect pollination. It is a simplified type and can be regarded as the basic form, not only of the Polygonaceae, but also of most of the higher Dicotyledons (Wodehouse, 1929). In this family it characterizes, with relatively little modification, the pollen of such entomophilous species as *Eriogonum*, *Triplaris*, *Antigonum*, *Fagopyrum*, and some species of *Polygonum*. Generally there are just three furrows, but in the pollen of some species there are always to be found, besides the ordinary tricolpate grains, some with larger numbers. Among the pollen of the species which we have before us, that of *Polygonum allocarpum* exhibits grains with 3, 4, 5, 6, 9, and 12 furrows, nearly always

[1] Tricolpate (τρις, three and κόλπος, fold) possessing three germinal furrows or harmomegathi.

[2] Harmomegathus (ἁρμόζω, accommodate, adapt; and μέγαθος, size) an organ for volume change accommodation, expansion fold.

beautifully arranged in the trischistoclasic [3] system or system of equal linear stresses already described for the grains of *Haplopappus* (Wodehouse, 1930.) The underlying principle of the arrangements of this system is that the furrows tend to be equal in length and to converge in threes with equal angles between them. The inevitable result of such an arrangement of furrows is that if there are six on a grain they must occupy the positions of the edges of a tetrahedron, nine of them the edges of a right triangular prism, and twelve of them the edges of a cube. Occasionally one or more furrows may be missing from any of these configurations without otherwise disturbing their arrangement, as, for example, in the 4- and 5-colpate grains. The possession of more furrows than the normal three is not alone confined to the pollen of *Polygonum allocarpum*, but is found here and there almost throughout the family and must be regarded as an inherent tendency, a vagary which, after all, is only an extension within the system of equal stress arrangements and characterizes the pollen of many unrelated Dicotyledons which have 3-colpate grains as the basic form.

An "edaphic" modification of this form of grain is found in the pollen of the anemophilous members, *Rumex*, *Rheum*, and *Muehlenbeckia*. The inevitable result of this mode of pollination is that their grains are thin walled, and its corollary that the furrows are much reduced, their harmomegathic function being unnecessary in association with a thin and flexible wall. In all of these the furrows are just linear grooves of negligible width even when expanded. When these grains dry their surface dips in along the furrows and they become deeply lobed, the furrows acting throughout their entire length as hinges for the bending of the thin exine. When the grains are moistened they become spheroidal, disclosing a small and weakly developed germ pore in the middle of each furrow. Owing to the hinge action of the furrows they are necessarily long, reaching almost from pole to pole when the grains are 3-colpate, or almost meeting each other at the centers of convergence when polycolpate, as is generally the case with the grains of *Rumex* and occasionally with those of *Muehlenbeckia*. If the reduction in the thickness of the exine had been carried a little farther it is quite conceivable that it would have resulted in the total disappearance of both pores and furrows, for they are organs of the exine. Indeed so near the vanishing point have they approached in the grains of some species of *Rumex* that it seems likely that such is the origin of the furrowless and poreless forms of grain that are encountered here and there among the pollen of wind-pollinated plants of other families; but in this family I have not found grains of this type entirely devoid of furrows.

A directly opposite line of development is found among the pollen grains of some of the members of this family which have retained insect pollination,

[3] Trischistoclasic (τρις, three; σχιστός, branching or parted; κλάσις, a breaking), the system of triradiate cracking in which the furrows of pollen grains tend to form, a system similar to the cracking of drying mud or shrinking plaster, as if produced by equilateral stresses.

as for example *Persicaria*, *Tracaulon*, and some species of *Polygonum*. In these not only has the exine remained thick, but it has become built up into an elaborate system of high vertical ridges anastomosing freely throughout, which, of course, imparts a stiffness to the exine causing the grain to be encased in a shell of rigid inflexibility accompanied by a loss of furrows and a great increase in the number of pores. The character of these grains has departed so far from the basic form that it would be difficult to see any relation between them, were it not for a few intermediate forms which clearly establish the connection. In the grains of *Polygonum californicum*, for example, part of the surface is alveolate and part of it is unmodified. Though only a part of the surface is involved, it includes the areas over both poles, the regions which must take up most of the bending when the furrows open and close, and consequently their harmomegathy is necessarily greatly impeded. Associated with this—probably as a consequence of it—these grains have very short and ineffective furrows. Moreover there is no compensating device to offset the reduction of the furrows; and I think that the reason for this is to be found in the size and shape of the grains for they are very small, measuring exclusive of the alveolate thickenings at the poles, $19.4 \times 12.5\ \mu$, the smallest in the family. Owning to the fact that in solid bodies volume is a function of the cube of the linear dimensions while surface area is function of the square of the same dimensions, the small size of these grains results in a relatively small ratio between their volume and their surface area, and this ratio is still further reduced by their elongate shape; consequently there is sufficient elasticity in their walls to accommodate whatever slight changes in volume there are, without any help from the furrows. In some ways this grain may be regarded as intermediate between the basic smooth type and the fully alveolate, and suggests the mode of origin of the latter from the former, though itself, in its small size, is divergent from both.

A more complete development of the alveolate structure is found in the grains of *Polygonum chinense*. Here the entire surface is covered with anastomosing upstanding ridges of such a character as to impart the utmost rigidity to the whole exine, which, it can readily be seen, would effectively prevent the opening and closing of any type of furrow, particularly the long tapering one which characterizes the basic form of grain of this family. These grains have three moderately long furrows, and it is obvious that their operation as expansion mechanisms is greatly impeded by the alveolate structure of the exine, for when the grains are fully expanded the furrows do not open wide throughout their full length but merely bulge out in the middle, pushed apart by the protrusion of the large germ pore, remaining pinched off toward their ends. These grains are large, $43.2\ \mu$ in diameter, which means that their volume is about twenty times as great as that of the grains of *P. californicum* while their area is only about nine times as great. As a consequence in this case some compensating device is necessary to make

up for the lack of harmomegathy of the furrows. This is apparently accomplished by an increase in the size of the pores. Perhaps the most remarkable thing about these grains is their large germ pores, measuring about 10.3 × 13.8 μ, and bulging prominently when the grain is moistened, thus affording a means of accommodating changes in volume, and taking over, to a large extent, the impaired harmomegathic function of the furrows.

In the grains of the Persicarias a still further advance in the same direction is found. Here the demobilization of the exine is complete, there remains no trace of the furrows, and the pores are each completely enclosed in a single lacuna; but in this case the loss of the furrows is abundantly compensated for by the large number of the pores. These cannot actually be counted but their distance apart, taken in consideration with their arrangement, permits one to estimate their number at about thirty. Their arrangement is plainly that which they would occupy if they were at the centers of furrows distributed over the surface in accordance with the system of linear stresses, an arrangement already familiar to us in this family in the grains of *Polygonum allocarpum*.

Briefly then it may be said that, in the evolution of these pollen-grain forms, the tendency to develop an alveolate structure, with its resultant stiffening of the exine and demobilization of the furrows, stimulated a compensating increase in the number of the pores, a tendency manifestly already inherent in the family, and this resulted in the development of the extraordinarily beautiful form of grain of *Persicaria* and allied genera, decorated with a continuous reticulum of high ridges and provided with about thirty pores. This is the culmination of this line of development in the family.

It is interesting to look ahead and see what the next step in such a line of development might be. There seems to be a universal tendency among the Angiosperms to abandon insect pollination and adopt wind pollination, a step which tends to induce a reduction of pollen-grain sculpturing. Nearly all wind pollinated Angiosperms are apparently derived from some ancestral form which was insect pollinated. This is reflected in the classification of living species of wind pollinated plants, for they are generally found in the same families with others which are insect pollinated; for example, the poplars in the willow family, the ragweeds and sage brushes in the composite family, and the ashes in the olive family. In every one of these instances the change from insect to wind pollination was accompanied by a reduction of, or entire loss of, surface decorations of the pollen grains.

There are no wind pollinated members of the Polygonaceae derived from ancestors with many-pored alveolate grains. (We have already seen that the forms of the grains of the wind pollinated *Rumex*, *Rheum*, and *Muehlenbeckia* were derived from the ordinary tricolpate form of grain). But the neighboring families Chenopodiaceae and Amaranthaceae are entirely wind pollinated, and consequently their grains are devoid of decorations. These

two families may not have been derived from the Polygonaceae, but a consideration of their grains in association with those of the Polygonaceae is suggestive of the form to which an extension of the evolutionary sequence of forms established in the Polygonaceae might lead, under the influence of wind pollination. The grains of the Chenopodiaceae and Amaranthaceae are cribellate (Wodehouse, 1928); that is to say, characterized by a large number of pores, giving the exine a sieve-like appearance. They are thin walled and the exine is devoid of decorations other than the numerous round germ pores. It is likely that the many-pored character of the grains of these two families was called forth in some ancestral species by some form of sculpturing which brought about the demobilization of the exine, but which has since been lost as the result of their adoption of wind pollination.

KEY TO THE SPECIES

A. Furrows present.

I. Furrows long and extremely narrow linear channels; exine thin.

a. Furrows always three; germ pores circular; texture fine-granular ..... *Rheum officinale*

b. Furrows three or four, generally both 3- and 4-colpate grains present; germ pores elliptical.

i. 26.5–32 μ in diameter, pores 4.6 μ long.

α. Texture granular ..... *Muehlenbeckia vulcanica*

β. Texture finely pitted.

1. Furrows generally three, rarely four or five ..... *Rumex crispus*

2. Furrows prevailingly 4, less frequently 3, and rarely 5 or 6 ..... *Rumex obtusifolius*

ii. Grains 18.2–23.9 μ in diameter.

1. Furrows prevailingly 4, less frequently 3... *Rumex Acetosella* (fig. 1)

2. Furrows prevailingly 3, less frequently 4... *Rumex Acetosa*

3. Furrows always 3 ..... *Rumex scutatus*

II. Furrows not linear, broader towards the middle, tapering towards their ends; exine thick.

1. With the entire surface conspicuously granular or pitted, or both. Germ pores circular or somewhat elliptical.

a. Grains over 40 μ in length, long-ellipsoidal; texture very coarsely granular and pitted throughout.

i. Grains long-ellipsoidal, furrow membranes heavily flecked; germ pore decidedly elliptical ..... *Fagopyrum esculentum*

ii. Grains nearly spherical; furrow membrane lightly flecked, germ pore somewhat elliptical or circular ..... *Antigonum guatamalense*

b. Grains less than 40 μ in length.

i. Bulging between the furrows, therefore tending to be triangular in cross section. Furrow margins raised; pore circular; Texture granular throughout ..... *Chorizanthe pungens* (fig. 2)
Chorizanthe Parryi

ii. Not bulging between the furrows; furrow margins not raised.

1. Grains 32–39.5 μ long; germ pore nearly or quite circular; texture fine pitted..... *Triplaris americana*
Triplaris caracasana
Triplaris filipensis

2. Grains 27.5–31 μ long.

α. Germ pores circular.

Furrow membranes flecked..... *Eriogonum gracile* (fig. 3)
Furrow membranes smooth..... *Eriogonum galioides*

β. Germ pore elliptical; furrow membranes smooth..... *Polygonum buxiforme*
Polygonum allocarpum

2. Chimaerical, with part of the surface granular and part alveolate..... *Polygonum californicum* (fig. 4)

3. With entire surface alveolate; furrows always three, short, not extending much beyond the pores; grains about 43 μ in diameter..... *Polygonum chinense* (fig. 5)

B. Furrows absent; entire surface alveolate; pores about 30, disposed over the surface in the trischistoclasic system; grains 45.5–70 μ in diameter..... *Persicaria Muehlenbergii* (fig. 6)
Persicaria pennsylvanica
Persicaria hydropiperoides
Persicaria Persicaria
Persicaria acuminata
Tracaulon arifolium
Tracaulon sagittatum

Rumex L. (Dock)

Grains, when moist, spheroidal or tending to be somewhat ellipsoidal, slightly bulging between the furrows; somewhat various in size in the different species; those of *R. crispus* and *R. obtusifolius* 18–32 μ in diameter. Furrows long, very slender, and pointed at their ends, varying in number from three to six, but generally three or four, arranged according to the system of linear stresses. When there are three they are equally spaced on the equator and directed meridionally; when there are six their arrangement corresponds to the six edges of a tetrahedron; when there are four they are in about the same position as when there are six, merely lacking from that configuration two furrows on opposite sides. In other words, in 4-colpate grains the four furrows are equally spaced around the equator, but are not directed meridionally as when there are three; instead they cross the equator obliquely and converge in pairs alternately above and below it. Furrow membranes smooth. Germ pores elliptical in shape with their long axes directed in the same sense as those of the furrows, small, 3.4–4.6 μ long, sharply defined. Texture of the exine always distinctly pitted, though of somewhat various coarseness in the different species. Normal grains always tightly packed with starch which shows plainly through the thin transparent walls of the grain.

Rumex is a genus of about 140 species of mostly homely herbs, of wide distribution. Taxonomically they are grouped in the two sections, ACETOSA and LAPATHIUM. To the former belong the sorrels, *R. Acetosella*, *R. Acetosa*, and *R. scutatus*, and to the latter the docks proper, *R. crispus* and *R. obtusifolius*. The slight genetic difference between these two groups is reflected to a certain extent in the dimensions of their pollen grains; those of the former group ranging, in the species here examined, from 18.2–23.9 μ in diameter with their germ pores uniformly 3.4 μ in diameter, while those of the LAPATHIUM series range from 26.5–32 μ in diameter with their germ pores 4.6 μ in diameter. The pollen of other species of these two groups should be examined to see if this difference holds for all and is really a character of sectional value.

All are wind pollinated, and some are counted as minor factors in hayfever. *R. hymenosepalus* Torr., Canaigre, is cultivated in California and is said to be an important cause of hayfever in San Fernandez valley (Selfridge, 1920). *R. conglomeratus* Murr. is stated by Hall (Schepperrell, 1917) to be of only minor importance in California, though abundant and producing much pollen. The pollen of the various species possesses all the physical characters of hayfever pollen and, in my experience, is caught on the pollen slides in fairly large quantities almost throughout the summer. The reason why it should be so much less toxic to hayfever patients than the pollen of the grasses, or even of plantain, is probably bound up with its chemical make-up and deserves further inquiry.

The grains of six species are described and illustrated by Meinke (1927) in connection with his studies on post glacial silts, but, as far as I am aware, they are seldom recorded from bog deposits; this is probably owing to the fragile nature of their exine which, in all the species of this genus, is very thin and tends to collapse beyond recognition when empty.

Rumex Acetosella L., Sour dock (Pl. LI, fig. 1). Type. Grains rather uniform in size, 22–24 μ in diameter. Furrows three, more frequently four, or occasionally six. Germ pores 3.4 μ long. Exine coarsely pitted, more so than in the grains of *R. crispus* and *R. obtusifolius*, about the same as in those of *R. Acetosa* and *R. scutatus*. These grains may be distinguished from the two latter, which they resemble most closely, in being prevailingly 4-colpate.

Low herbs in dry fields and waste places. Wind pollinated, shedding large amounts of light air-borne pollen, June and July; throughout North America, except in the extreme north; introduced from Europe. Generally speaking rather an unimportant factor in hayfever. Said to be important in California (Rowe, 1928; Selfridge, 1920) and in Oregon (Chamberlain, 1927).

Rumex Acetosa L. Green sorrel. Sour or sheep dock. Grains essentially as in the type, rather uniform in size, 18.2–21.6 μ in diameter. Furrows 3, or, less frequently, 4. Germ pores 3.4 μ long.

A low herb, similar to though much larger than the preceeding. Intro-

duced from Europe or Asia, now widely distributed in the United States and Canada, wind pollinated, shedding rather large amounts of pollen in early summer. Not known to be a factor in hayfever but probably not distinguished in hayfever studies from the preceding species.

Rumex scutatus L. Roman sorrel, Oseille ronde. Grains spheroidal, uniform, 23.9–25.1 μ indiameter, furrows nearly always three, texture more finely pitted than in the two preceding species. Otherwise as in the type. A low weed widely distributed in Europe; wind pollinated but not known to be a cause of hayfever.

Rumex crispus L. Curled, or Yellow dock. Grains spheroidal, rather uniform in size, 28.5–32.0 μ in diameter. Furrows three, or rarely four or five; germ pore 4.6 μ in length. Otherwise as in the type. These grains may be distinguished from those of the three preceding species by their larger size, and from those of *R. obtusifolius*, which they resemble more closely, by being prevailingly tricolpate, and with a somewhat finer texture to their exine.

Tall rank herbs, roadsides and waste places almost throughout North America; wind pollinated, but shedding relatively little pollen and constituting, outside of a few restricted localities, only a minor cause of hayfever. It is, however, regarded as important in Oregon (Chamberlain, 1927). In California it is regarded as unimportant by Hall (Schepperrell, 1917) but important by Selfridge (1920). In my experience in the vicinity of New York City it sheds too little pollen ever to become a serious factor in hayfever.

Rumex obtusifolius L. Broad-leaved or bitter dock. Grains spheroidal in shape, somewhat various in size, 26.4–31 μ in diameter. Furrows prevailingly four, less frequently three or six, rarely five. When more than three they are somewhat variously united or irregular in arrangement. Germ pores 4.6 μ long. Otherwise as in the type. This grain may be distinguished from that of *R. crispus*, which it resembles most closely, by being prevailingly tetracolpate and of a slightly more coarsely pitted texture.

A rank weed of roadsides and waste places. Similar in habit and appearance to the preceding. Regarded as a minor cause of hayfever in California (Hall in Schepperrell, 1917).

Rheum officinale Baillon. Thibetan rhubarb. Grains similar to those of *Rumex Acetosella*, spheroidal or ellipsoidal, rather uniform, about 30 μ in diameter; tricolpate with furrows long and very slender, sunken in the surface of the grain to such an extent as to give it a three-lobed shape; each is provided with a single small round or nearly round pore. Exine somewhat heavier and pitting finer than in the type (*Rumex Acetosella*).

Native of Thibet and Western China. Occasionally cultivated as a foliage plant, and the source of at least part of officinal rhubarb. The genus *Rheum* L. includes about 20 species, native of Asia, from Siberia to the Himalayas and Palestine. The best known species is the common garden rhubarb or wine-plant (*R. Rhaponticum* L.)

Muehlenbeckia vulcanica (Benth.) Endl. Grains resembling those of *Rumex Acetosella*, spheroidal or oblately flattened, 26–27.5 μ in diameter; texture granular with the granules fused so as to form a more or less continuous reticulum. Otherwise as in the type.

The genus *Muehlenbeckia* comprises about fifteen species of small shrubs and woody climbers, natives of Australia, New Zealand, South America, and the Solomon Islands. The flowers are not showy and are unisexual, monoecious or dioecious; they therefore appear to be wind-pollinated, though I have not been able to determine this point with certainty. Among them are such well known cultivated plants as the Wire-plant (*M. complexa* Meissn.), a creeper from New Zealand, much cultivated in California and elsewhere; and the curious Centipede-plant (*M. platyclados* Meissn.), a curiosity of the greenhouse, from the Solomon Islands.

Eriogonum Michx. (ERIOGONUM, UMBRELLA PLANT)

Grains uniform in shape and size, ellipsoidal, about 32 × 25 μ; tricolpate with long tapering furrows which gape rather widely open when the grain is moist, disclosing the circular germinal aperture through which the pore bulges more or less prominently. The furrow membrane is for the most part smooth though occasionally it may be lightly flecked with a few granules which tend to occupy a strip along its center. Exine thick, slightly thicker along the margins of the furrows and along three meridional strips between the furrows, recalling the thickenings in the grain of *Chorizanthe* (Pl. LI, fig. 2) but to a lesser extent; texture distinctly and rather coarsely granular with the granules in the vicinity of the germ pores tending to be arranged in rows resembling thumb-print markings, but unorganized in other parts of the grains.

These grains differ from those of the foregoing genera in their heavier exine and consequently better development of their furrows. The plants are insect-pollinated, while those of *Rumex* and *Rheum*, and probably *Muehlenbeckia*, are wind-pollinated. Thus we see, in the comparison of the grains of these two groups, an expression of an almost universal law—that when pollen becomes dispersed by wind the exine becomes thinner. This form of grain may be regarded as the least specialized in the family, the form from which the others were probably derived.

The genus comprises over two hundred species of low herbs, natives of America, mostly of the western states. All are insect pollinated, and none is regarded as a cause of hayfever.

Eriogonum gracile Benth. (fig. 3). Type. Grains as in generic description, furrow membrane occasionally slightly flecked. Dry plains, valleys and low hills: Great valley and Coast Ranges to southern California and Lower California.

Eriogonum galioides I. M. Johnston. Grains as in generic description, furrow membrane smooth. Low prostrate perennial herb, common on San Louis Island, Gulf of California.

Chorizanthe R. Br.

Grains similar to those of *Eriogonum*, somewhat ellipsoidal, about 30 × 25 μ, tricolpate with rather short tapering furrows with their margins conspicuously raised forming prominent ridges. Germ pore clearly defined, circular; furrow membrane smooth. The grain bulges quite conspicuously between the furrows, thus giving it something of a triangular shape in optical section (fig. 2). Exine thick and very coarsely granular, but granules without lineal arrangement. These grains differ from those of *Eriogonum* in the greater extent of their intercolpar thickenings, their more coarsely granular texture, and their shorter furrows.

About 30 species in western North America and Chili. Low dichotomously branching desert herbs.

Chorizanthe pungens Benth. (fig. 2). As in generic description, indistinguishable from the following species. Low prostrate herb, common in San Francisco Bay region of California and southward.

Chorizanthe Parryi Wats. As in the generic description. Low flat-topped plants 3–15 inches broad, southern California, common on gravelly mesas near Crofton, San Bernardino County.

Triplaris Löfl.

Grains ellipsoidal, or nearly or quite spheroidal when fully expanded, tricolpate with furrows long and tapering but narrower than in *Eriogonum*. Germ pores circular or slightly elliptical. Furrow membranes smooth. Exine rather thick but various in the different species, more or less granular and distinctly but finely pitted. These grains differ from those of *Eriogonum* in their more nearly spheroidal shape, thinner exine without thickenings between the furrows or along their margins, and in the fine pitting of the exine, in all of which characters they tend to resemble *Rumex*.

A genus of about ten species of small trees, tropical South America.

Triplaris americana L. Grains uniform, spheroidal or short-ellipsoidal, about 35.3 × 33.1 μ, always tricolpate. Otherwise as in the generic description.

Native of South America, occasionally cultivated under glass, or out-of-doors in the Southern States.

Triplaris felipensis Wedd. Grains uniform, spheroidal when expanded, 35.3–36.5 μ in diameter. Otherwise as in generic description. Colombia, South America.

Triplaris caracasana Cham. Grains uniform, spheroidal, 31.4–34.2 μ in diameter. Pitting of the exine finer than in *T. americana*. Otherwise as in the generic description. Venezuela. Flowers in May.

Fagopyrum esculentum Moench. Buckwheat. Grains long-ellipsoidal in shape, extremely various in size, but the majority of them measuring about 57 × 47 μ; tricolpate with furrows long and tapering; germ pores not sharply defined but decidedly elliptical; furrow membranes conspicuously

flecked with large coarse granules. Texture conspicuously pitted and with a coarse underlying granular structure.

A low annual herb, native of Europe and northern Asia, much cultivated elsewhere and frequently escaped. June to Sept. Insect pollinated.

Antigonum guatamalense Meissn. Grains short-ellipsoidal to spheroidal, 57–62 μ long, 3-colpate with rather long tapering furrows. Germ pores sharply defined, circular or slightly elliptical. Furrow membranes lightly flecked with a few loose scattered granules. Exine thick and rugged with a coarse granular and pitted texture. This grain may be distinguished from that of *Fagopyrum* by its more nearly spherical shape, coarse texture, and less conspicuously flecked furrow membranes. Woody climbers in Guatamala.

The genus *Antigonum* contains three or four species of tendril-climbing vines, in Mexico and Central America. One species, the Coral Vine or Corallita, is cultivated in the Southern States for the beauty of its bright pink flowers.

Polygonum (Tourn.) L. (KNOTWEED, JOINTWEED)

Several very different forms of grain are found among the species of this genus, and these exhibit characters both of the preceding genera and of the very different grains of the succeeding genera. The basic form of grain of *Polygonum* is tricolpate with long tapering furrows, heavy granular exine, and decidedly ellipsoidal shape, essentially the same as that of *Eriogonum* (fig. 3) except that it lacks the intercolpar thickenings of the exine and the thickenings along the margins of the furrows of the latter. This form of grain is exhibited, among those members of this genus which we have before us, by *P. buxiforme*. I have not been able to examine the pollen of more than a very few species and therefore cannot say from personal observation if this form really does characterize the grains of the majority of species. Nevertheless Fischer (1890) finds that all ten of the species that he examined "haben drei parallele Falten mit je einer kreisrunden Keimpore; die Exine ist doppelt, die Zwischenstäbchen an den Polen feiner und dichter, an den Seiten dicker und weitläufiger gestellt; Oberfläche fast glatt." It therefore seems safe to assume that such is the basic form of the pollen grains of *Polygonum*.

This simple form undergoes, in some of the species, two highly suggestive modifications. The first is the rather remarkable multiplication of furrows recalling the condition in *Rumex* and others, as for example in the grains of *P. allocarpum*. In the grains of this species the furrows are beautifully symmetrical, and occur in most of the theoretically possible arrangements of the trischistoclasic series of configurations up to that requiring twelve furrows, and it is likely that an examination of the pollen of other species, or possibly a more extended examination of the pollen of this species, would discover some grains with higher numbers of furrows in the same system.

The other modification that the simple basic 3-colpate grain undergoes in this genus is the development of an alveolate structure of its exine. This is partly expressed in the grains of *Polygonum californicum* in which the alveolate and simple granular characters are combined in chimærical fashion. It reaches its fullest expression, however, in the grains of *P. chinense*, in which the entire surface is alveolate, in this respect resembling the grains of *Persicaria*.

Polygonum buxiforme Small. Shore knotweed. Grains uniform ellipsoidal, averaging about 28 × 23 μ, furrows 3, occasionally six, long and tapering, deeply depressed giving the grains a lobed appearance. Furrow membrane smooth, germ pore elliptical with its long axis directed in the same sense as that of the furrow. Exine thick with texture distinctly but finely granular. These grains are similar to those of *Chorizanthe* and *Eriogonum*, but do not bulge between the furrows as in the former, and their germ pores are distinctly elongate instead of circular as in the latter.

A prostrate bushy weed, on shores and in waste places, almost throughout the United States and Canada. Aug.–Sept.

Polygonum allocarpum Blake. Grains essentially as in *P. buxiforme*, except that the majority have six furrows, relatively few have three, and occasionally grains with 4, 5, 9, and 12 furrows are found. In nearly all of these the furrow configurations are beautifully regular, conforming almost exactly with the series described for *Haplopappus* (Wodehouse, 1930). The grains are uniform in size 28.5–29.6 μ in diameter.

Branching annual herb, sea coasts and adjacent islands, Maine and New Brunswick.

Polygonum californicum Meissner (fig. 4). Grains uniform in size and shape, ellipsoidal, 19.0 × 12.5 μ. The exine of the two ends and three longitudinal strips alveolate, almost as in *Persicaria*, but over three large elliptical areas surrounding the three furrows the exine is granular as in the grains of *Polygonum buxiforme* and *P. allocarpum*. Furrows short and tapering, furrow membranes smooth, germ pore elliptical. This grain combines the characters of those of *Persicaria* and *Polygonum*, in such a way that it may truly be spoken of as chimærical, and suggests the mode whereby one form could have been derived from the other.

A slender wiry annual, diffusely branching, 3–7 in. high. Dry foothills, Sierra Nevada and North Coast Ranges; north to Washington. July.

Polygonum chinense L. (fig. 5). Grains uniform, spheroidal, about 43.3 μ in diameter, tricolpate with furrows short and sharply tapering, furrow membrane smooth, germ pore large, elliptical, occupying nearly the whole furrow. The entire surface of the grain is marked off into angular lacunae by a reticulate system of high ridges. In this respect the grain is almost exactly like those of *Persicaria*. The ridges have the appearance of being composed of vertical prisms partially fused together, making irregular, jagged, palisade-like partitions between the lacunae. The floors

of the lacunae are studded here and there with a few little bead-like thickenings. The alveolar system abuts upon the furrows with mostly closed lacunae, showing that the furrows are not torn through the reticulum but are developed simultaneously with it as coördinate structures.

This form of grain is curiously intermediate between the basic form of the genus and the furrowless alveolate form of *Persicaria*, but tends more towards the latter. In its large size, spheroidal form, and alveolate surface it is persicarioid, but in its possession of three furrows with three germ pores it is polygonoid.

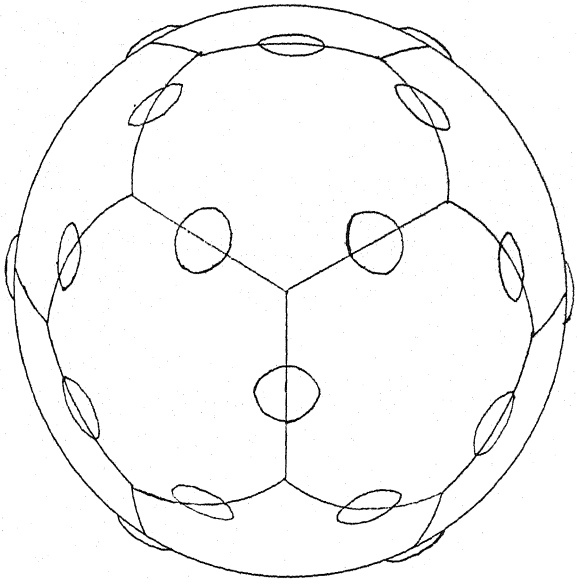
A shrubby perennial, about 5 ft. high with white, pink, or purplish flowers in small panicled heads. Native of the Himalaya region and Ceylon to China and Japan, and the Philippines. Occasionally cultivated.

Persicaria (Tourn.) Mill.

Grains spheroidal, rather large, ranging from about 51 μ to about 70 μ in diameter; alveolate with the whole or part of the surface marked off into angular lacunae by a reticulate system of well defined ridges. The texture of the floor of the lacunae is more or less pebbled, always coarse but various in the different species. The pebbled structure also involves the ridges which are extremely rough and jagged as a consequence, presenting the appearance of a breakwater built of closely packed but crookedly driven piles. Elements similar to those which go to make up this remarkable structure are encountered, in a lesser degree of development, in the granular and pitted texture of the grains of *Fagopyrum* and *Antigonum* and, to a lesser extent, of *Rumex;* so this structure, remarkable as is its appearance, cannot be said to have arisen *de novo* in this genus or in *Polygonum* but is in reality the culmination of a tendency variously expressed in the grains of most of the other species of the family.

Furrows are entirely absent in all species here considered; the germ pores are set here and there in the ordinary lacunae of the reticulum, and the lacunae occupied by them are frequently difficult to distinguish from those that are unoccupied, but may generally be recognized by their smaller size, and their smooth and slightly bulging floor. The system governing the arrangement of the pores is not readily apparent, because in all the species which we have before us the pores are round, and consequently do not divulge their spatial orientation in the way that furrows do. It can readily be seen, however, that their arrangement is not isometric as would be suggested by their rounded form; for if one pore is regarded as a center of a group it is found not to be surrounded by five or six others at equal distances as would be the case if their arrangement were isometric. Instead they are arranged according to the system of equal linear stress or trischistoclasic system, the arrangement which governs most furrow configurations. In these grains if one pore is regarded as a center it is found to be flanked by two pairs of pores which are equidistant and on opposite sides (text fig. 1). The pattern

of the arrangement of the group of five pores thus obtained, resolves itself into two equilateral triangles in which the initial or central pore of the group serves as the apex of both; and we find that each of the three pores of each triangular group is likewise the member of a neighboring triangular group, thus forming a continuous system throughout. The characteristic whereby this system may be recognized is the repetition of the triangular group.



TEXT FIG. 1. Diagram of the pore arrangement of a *Persicaria* pollen grain with thirty pores arranged in the trischistoclasic system. The lines, arcs of great circles, represent the axes of hypothetical furrows. These form a continuous triradiate system which, when carried to completion over the surface of the sphere, results in the formation of twelve spherical pentagons with thirty interfacial boundaries, corresponding in arrangement to the twelve faces and thirty edges of a pentagonal dodecahedron. The pores are represented as circles at the centers of the lines, or hypothetical furrow axes.

The circular form of the pores of the species of *Persicaria*, which we have before us, makes it surprising that their arrangement should be according to that of the trischistoclasic system, one which is generally associated with elongate furrows, rather than circular pores. Much light, however, is thrown on their origin by a consideration of the grains of *Persicaria amphibium* which, according to the description of Fritzsche (1837) and his beautiful figure, are provided with thirty furrows, marking off the surface of the grain into 12 pentagonal areas corresponding exactly to the twelve faces of a pentagonal dodecahedron. If, in such a grain as this, the thirty elongate furrows were to be replaced by as many round germ pores their arrangement would be exactly like that of the various species of *Persicaria* which we have before us (except that the number of pores is not always

thirty) and I have no doubt that such is the explanation of the peculiar arrangement of these pores. They should be regarded as shortened furrows, derived from a many-furrowed grain in which the arrangement of the furrows followed the ordinary trischistoclasic system. The cause of the shortening was probably the extraordinary development of the reticulate pattern with its resultant demobilization of the exine. The large number of pores possessed by these grains (about 30) abundantly compensates for the loss of the harmomegathic action of the furrow, for changes in volume are readily accommodated by the bulging or invaginating of so many pores.

A large genus of about 125 species of annual or perennial herbs. Prince's Feather (*P. orientalis* (L.) Spach) is a familiar example in cultivation.

Persicaria Muhlenbergii (S. Wats) Small. Swamp Persicaria (fig. 1). Type. Grains rather uniform in size about 57 μ in diameter, ridges of the reticulum heavy, apparently composed of more or less vertical prisms. The floors of the lacunae thickly and coarsely pebbled. Otherwise as in the generic description. In swamps and moist soil almost throughout North America. July to Sept.

Persicaria pennsylvanica (L.) Small. Pennsylvania Persicaria. Grains uniform, 68–74 μ in diameter, essentially as in the type, except that the ridges are a little heavier. Nova Scotia to Ontario, Minnesota, Florida, and Texas. July–Sept.

Persicaria hydropiperoides (Michx.) Small. Wild water-pepper. Grains rather uniform, essentially as in the type about 45.6 μ in diameter.

In swamps and wet soil. Almost throughout the United States and adjoining parts of Canada.

Persicaria Persicaria (L.) Small. Lady's thumb, Heartweed. Grains uniform, 43–51 μ in diameter, essentially as in the type.

In waste places throughout North America, except the extreme north. Naturalized from Europe. June to October.

Persicaria acuminata (H. B. K.) Grains uniform about 51.3 μ in diameter. Essentially as in the type. In moist shady places almost throughout South America.

Tracaulon arifolium (L.) Rap. Halberd-leaved tear-thumb. Grains essentially as in *P. Muhlenbergii*, uniform, 51.3 μ in diameter. A slender reclining perennial herb with prickly stem, in moist places. New Brunswick and Ontario to Minnesota and south to Georgia. July–Sept.

Tracaulon sagittatum (L.) Small. Arrow-leaved tear-thumb. Grains as in *Persicaria Muhlenbergii*, uniform, about 57 μ in diameter. Plants similar to the preceding except for the arrow shape of the leaf.

I am indebted to the New York Botanical Garden for most of the material used in this study, and take pleasure in thanking Dr. E. D. Merrill, Director-in-Chief, for placing at my disposal the herbarium and laboratories.

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EXPLANATION OF PLATE LI

All figures were drawn from studies made at a magnification of 900, using a Zeiss 3 mm. apochromat objective, N.A. 1.4, and paired 15 × binocular eye pieces. Measurements were made at a magnification of 1800, using a 2 mm. apochromat objective, N.A. 1.3, and a 20 × eye piece. The sizes of the drawings are chosen to display best the characters of the grains and do not bear a constant relation to their sizes. The measurements given are those of average dimensions.

FIG. 1. Pollen grain of *Rumex Acetosella* with four furrows, shown in polar view, 23 μ in diameter. The furrows converge in pairs towards two centers in the upper hemisphere. Crossing the equator obliquely, they change partners and converge towards two other centers in the lower hemisphere, which alternate with those of the upper. The tricolpate grains are similar, but their three furrows are meridionally arranged, converging towards the two poles.

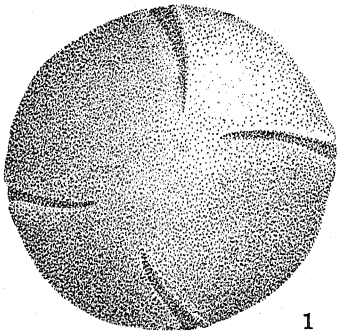
FIG. 2. Pollen grain of *Chorizanthe pungens*. Optical section showing the characteristic intercolpar thickenings of the exine, and the triangular outline of the grain. About 25 μ in diameter.

FIG. 3. Pollen grain of *Eriogonum gracile*, 32 × 25 μ, side view, showing the heavy exine and well developed furrows.

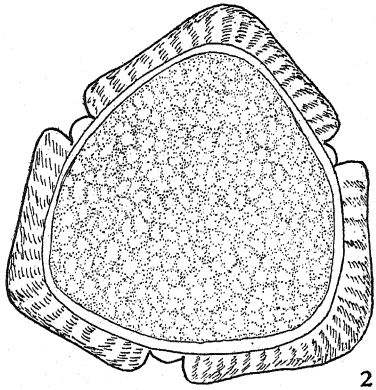
FIG. 4. Pollen grain of *Polygonum californicum*, 19 × 12.5 μ, showing the dual nature of its exine, part of which is granular like the grains of most other Polygonums and part of which is alveolate like those of *Persicaria*.

FIG. 5. Pollen grain of *Polygonum chinense*, 43.3 μ in diameter, showing the demobilization of the furrow by the alveolate structure of the exine.

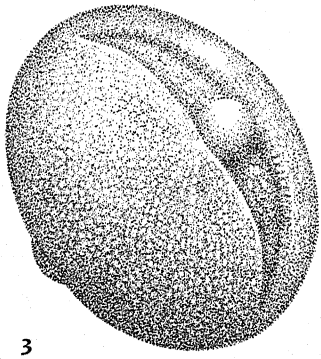
FIG. 6. Pollen grain of *Persicaria Muehlenbergii*, 57 μ in diameter, showing the alveolate structure of the exine and large number of small germ pores arranged in the trischistoclasic system.



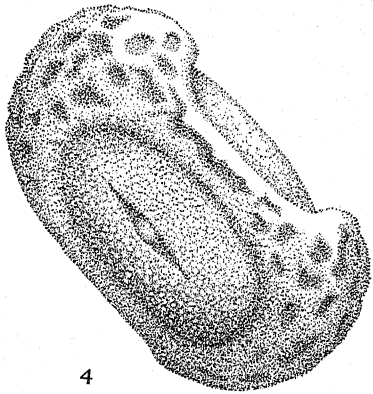
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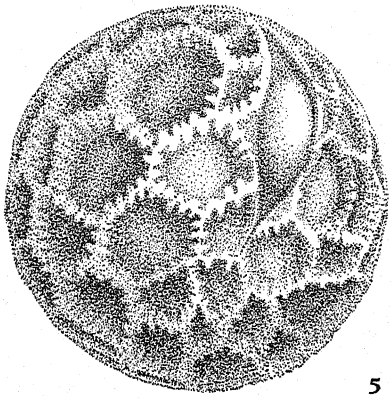
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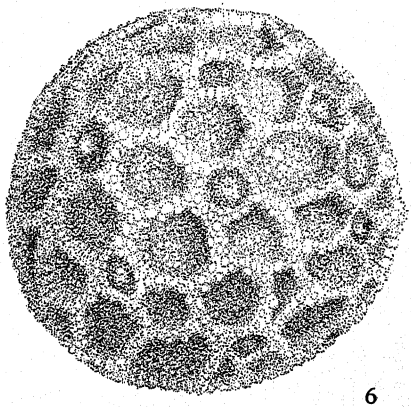
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CERTAIN PHASES OF CITRUS LEAF TRANSPIRATION [1]

E. T. BARTHOLOMEW

(Received for publication May 25, 1931)

The results of some of the previous work on the problem of citrus leaf transpiration indicate that the amount of water that escapes from the dorsal side of the leaf in which there are no stomata is relatively very great, and that the stomata become functionless very early in the life of the leaf. Coit and Hodgson (4) reported that ". . . from 40 to 50 percent of the water loss from citrus leaves occurs through the upper epidermis which does not contain stomata. These studies have shown that the young leaves are more efficient than the older leaves, but that even the youngest leaves lose as much as 25 percent of their water through the upper epidermis." With reference to stomatal movement they reported that: "By the use of Lloyd's method the amplitude of stomatal movement was studied. It was found that very early in the life of the leaf the stomata lose their power of opening and closing and remain practically closed thereafter. In some cases the closure is not complete and the stomata remain slightly open." Reed and Bartholomew (9), in connection with their study of the effects of desiccating winds on citrus, published some results on the comparative rates of water loss from the dorsal and ventral sides of detached leaves which appeared to confirm the results previously obtained by Coit and Hodgson.

Up to the present time comparatively little work has been done on the transpiration of citrus leaves. In addition to the work already mentioned, that of Boeuf and Genet (3), in connection with their study of the effects of the autumn sirocco on orange foliage, and that of Oppenheim (8) should be mentioned. These two studies, however, were concerned with the comparative rates of transpiration of different varieties of citrus and not with the comparative rates of water loss from the dorsal and ventral sides of leaves of different ages, as is the case with the present paper.

It is well known by all who have worked in this field that valid objections can be raised to each of our present methods of measuring transpiration, either quantitatively or comparatively, unless it be in the case of small potted plants that can be weighed. The author's method, which consisted of the use of calcium chlorid, is no exception. However, it is believed that the results are reasonably reliable since the main object of these tests was not so much to determine actual amounts of transpiration as it was to investigate such problems as (*a*) the comparative resistance of the dorsal and ven-

[1] Paper No. 249, University of California Graduate School of Tropical Agriculture and Citrus Experiment Station, Riverside, California.

tral sides of the citrus leaf to the loss of water, (*b*) the comparative amounts of water loss from the leaves as influenced by their age and variety, and (*c*) the amplitude and time of day of stomatal movement as indicated by the magnitude of water losses.

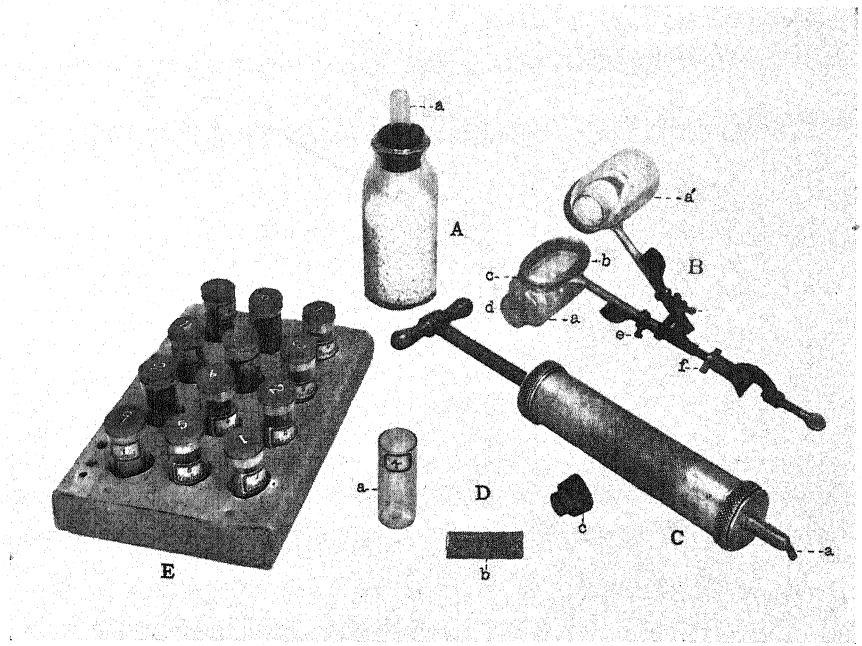
Materials and Method

The leaves tested were principally those of the Eureka lemon (*Citrus limonia* Osbeck) and the Valencia orange (*C. sinensis* Osbeck) but many tests were also made on leaves of the navel orange (*C. sinensis*), Marsh grapefruit (*C. paradisi* Macf.), and sweet lemon (*C. limonia*). All leaves were tested while attached to the trees, and all tests were made under field conditions. With the exception of the sweet lemon, which was a little older, all of the trees whose leaves were tested were four years old from the bud. Both dorsal and ventral sides of over 1500 leaves of different ages were tested. The age of the leaf is indicated by the growth cycle during which it was produced. The citrus tree normally produces three cycles of growth during the growing season: one in the spring, one in midsummer, and one in late summer or early autumn. Leaves from each of five different cycles were tested; that is, leaves from only a few weeks to nearly two years old. Citrus leaves on a given branch are considered to be mature by the time a new growth cycle begins to develop. The citrus leaf has stomata in its ventral surface only.

The method used in making these tests was similar to the one used by Bews and Aitken (2), and by Heinicke (5), but the apparatus was different. The former used small glass tubes half-filled with calcium chlorid and plugged with "cotton-wool" to keep the calcium chlorid in place during operation. The tubes were attached to the ends of a circular band spring, the spring serving to keep the open ends of the tubes pressed firmly against the two sides of the leaf while the tests were being made. Heinicke placed the calcium chlorid in weighing bottles which were held in place by modified wire test-tube clips.

The apparatus used by the author is shown in text figure 1. The calcium chlorid (*A*) was rescreened so that the granules would pass through an 8-mesh but not through a 12-mesh screen, and was kept constantly dry in an oven at 65° C. All calcium chlorid that appeared to have deliquesced during the test period was discarded, and none was used that had not been in the oven for at least six hours. The lower end of a small test tube was inserted in the rubber stopper of the bottle (*A a*) to serve as a measuring vessel. This vessel filled level full held approximately 2.25 grams of calcium chlorid. The calcium chlorid was used as required from the supply bottle as soon as it was taken from the oven, without waiting for it to cool. A towel was kept wrapped around the bottle so that it might be handled without discomfort and at the same time retain its heat as long as possible. When the measuring tube (*A a*) had been filled it was emptied into an 18-

mesh brass gauze basket (*D b*) and the rubber stopper (*D c*) inserted. The basket of calcium chlorid and the rubber stopper were then inserted into a small glass vial (*D a*) which served as a weighing bottle. This process was repeated until the desired number of vials had been filled, usually twelve, and then each was weighed to the third decimal place. Text figure 1 *E* represents a group of these vials, containing baskets of calcium chlorid, ready to be taken to the field, in a container made by wrapping corrugated pasteboard around two light blocks of wood, one block at each end.



TEXT FIG. 1. Apparatus used in measuring water loss from citrus leaves. *A*, calcium chlorid bottle with measuring tube; *B*, transpiration shell apparatus; *C*, grease gun filled with plastic; *D*, calcium chlorid absorbing and weighing apparatus; *E*, calcium chlorid vials in carrying container. See text for full description.

Text figure 1 *B* consists of an ordinary burette clamp, a pair of aluminum castings, and a pair of specially blown glass shells. The aluminum casting has a solid cylindrical arm 7.5 cm. long and 6 mm. in diameter, and an oval-shaped part with a wall 8 mm. deep and 7 mm. thick. The opening in the oval is 2.5 × 5.8 cm. In the edge of the oval at *b* there is a broad groove 3.5 mm. deep, and in the other edge, at *c*, a similar groove 2 mm. deep. The special glass shells (*B a a'*) are blown from ordinary white glass and the walls are 1 to 1.5 mm. thick. The circular opening at *d* is of the same diameter as that of the opening of the vial (*D a*) so that the large end of the rubber stopper (*D c*) will fit both of them. In one side of the shell there is an oval

opening whose dimensions are such that its 3 mm. band or collar in which it terminates will rest in the center of the shallow groove (*B c*) of the aluminum casting. The narrow collar surrounding the oval opening facilitates the cementing of the shell to the casting. For this purpose a cement called "china patch" was found to be very satisfactory.

It is evident that the object in using a pair of the shells for each leaf was to test the comparative resistance of the dorsal and ventral sides of the leaf to water loss. Six leaves were tested at a time. In order that the metal of the casting should not touch the leaf a set-screw (*B e*) was so adjusted that it held the ovals about 3 mm. apart. Before leaving the laboratory a plastic wax was run into the groove (*B b*) of each casting. It was found that this could be most easily done by using an ordinary grease gun (*C*) having a short tube with an opening of approximately 5 mm. After running the wax into the groove the finger was used to press the wax slightly toward the outer edge of the groove so that when the clamp was closed on to the leaf none of the wax would be forced out over the portion of the leaf to be tested.

In order to make an air-tight seal between the leaf and the apparatus and at the same time not injure or otherwise affect the leaf tissues, many different kinds of seals, such as very soft rubber and different kinds of waxes and plastics, were tried. The seal finally used was composed of approximately 100 parts resin, 100 parts beeswax, and 110 parts of purest white petroleum oil (viscosity 195 seconds Sayboldt). It was found advisable to vary slightly the proportions of the ingredients, depending on the temperature at the time the tests were being made. It was also found that the mixture worked best if, after melting the ingredients together, they were stirred or whipped, while cooling, until the mixture became viscous and light tan in color. At this stage it was packed into the grease gun.

The procedure for making tests with the apparatus which has just been described was as follows: When the grooves of the oval castings had been filled with wax and the calcium chlorid weighings had been made, the entire apparatus was taken to the field. The desired leaves were then chosen and iron supports driven into the ground at the proper distances from them. One or more clamps were attached to a given support, depending upon the location of the leaves. After *B* was attached to the support, the other end of the apparatus was clamped to the leaf by means of the thumb nut (*g*). After this had been done the two shells were lightly pressed together, thus insuring a perfect seal. The shells spring slightly outward as the pressure is released because of their distance from the set-screw contact. This action is not sufficient to impair the seal and is of importance because it frees the leaf tissues of practically all pressure. The swivel joint in the clamp (*B f*) made it possible to attach the shells to the leaf without materially changing its position, thus making it unnecessary to bend the petiole which might affect the water supply of the leaf. When the shells had been

attached to all of the leaves to be tested, the gauze baskets containing the calcium chlorid were transferred from the weighing vials to the shells where they remained for the desired length of time. At the end of the period, the baskets of calcium chlorid were transferred back to the weighing vials, brought to the laboratory, and reweighed.

Tests showed that the error due to the amount of moisture absorbed from the air while the transfers were being made, and due to the amount of moisture in the weighing vials and the shells attached to the leaves, was small. The amount of moisture absorbed by the calcium chlorid in this manner ranged from a fraction of a milligram to three or four milligrams, depending upon the humidity of the atmosphere at the time the tests were being made. During the day a cheesecloth shade was used to prevent the direct rays of the sun from striking the glass shells.

Records of temperature, humidity, sunshine, and wind velocity were kept for each period during which a test was made. The weather instruments were approximately fifty rods from the locations in which the leaf tests were made.

Tests and Results

Table 1 records the actual rates at which the calcium chlorid absorbed water from the dorsal and ventral sides of individual lemon and orange leaves. The tests on the lemon leaves were made on May 28 and 29, 1930, and on the orange leaves August 4 and 5.

A given citrus leaf may be more efficient or it may be less efficient in resisting the loss of water than the adjacent or nearby leaf on the same branch. The leaves of the Eureka lemon appear to be less variable in their resistance to the loss of water than those of the Valencia orange. Table 1 shows that the greatest difference between the maximum and minimum losses from the lemon leaves was 23 mgms. for the dorsal side and 292 mgms. for the ventral side; while for the orange leaves the differences were 61 and 468 mgms., respectively. Some groups of leaves showed differences which were even greater than these, while others showed differences that were much smaller.

The following computations from the data in table 1 are of interest. They show that of the total amount of water absorbed from the leaves, during the combined day and night test periods (15 hours), the relative proportions for the two periods were:

	Day Period (5 hours) Percent	Night Period (10 hours) Percent
From lemon leaves—Dorsal side	67	33
Ventral side	95	5
From orange leaves—Dorsal side	70	30
Ventral side	91	9

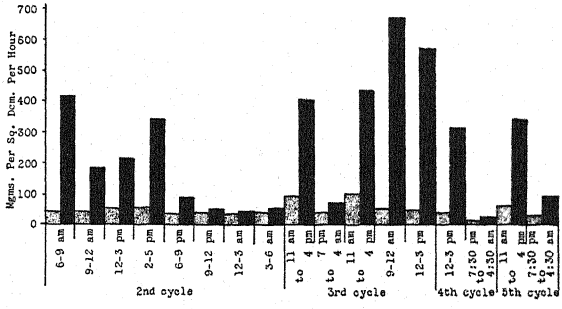
The next series of tests was on orange leaves and the results are given in text figure 2. A different lot of leaves was used during each test period,

TABLE 1. *Different Amounts of Water Absorbed by Calcium Chlorid from Adjacent or Nearby Leaves on the Same Branch*

Number of Leaf*	Increase in Weight of $CaCl_2$, Milligrams per Square Decimeter per Hour			
	10 A.M. to 3 P.M. (5 Hours)		7 P.M. to 5 A.M. (10 Hours)	
	Dorsal	Ventral	Dorsal	Ventral
Eureka lemon leaves; fourth growth cycle; 30 leaves on cycle**				
17	53	658	24	33
18	50	600	22	30
20	63	838	32	45
21	67	745	33	39
24	73	892	37	43
26	60	862	30	63
Average....	61	766	30	42
Percent....	7	93	42	58
Valencia orange leaves; fourth growth cycle; 35 leaves on cycle**				
18	83	852	32	59
19	72	701	28	39
20	72	732	28	63
25	100	596	50	67
28	61	384	28	74
29	122	462	50	56
Average....	85	621	36	60
Percent....	12	88	37	63

* The numbers in this column denote the position of the leaves on the growth cycle; the smaller the number the younger the leaf.

** The oldest of the last four cycles of growth.



TEXT FIG. 2. Comparative rates of water loss from Valencia orange leaves, on different growth cycles, during different portions of the day and night, and from the dorsal and ventral surfaces, of the leaves. Dotted columns denote the loss from the dorsal surface, solid columns the loss from the ventral surface.

and each lot of leaves was not only on a different branch but often on a different tree.

The times when tests were made and the approximate ages of the leaves on the different cycles were as follows:

Cycle	Months Tested	Approximate Age of Leaves
Fifth	May	20 months
Fourth	August	14 months
Third	May and August	11 months
Second	July and August	2 months

The results recorded in text figure 2 show not only the relative amounts of water absorbed from the dorsal and ventral sides of orange leaves but also the relative amounts absorbed, during the day and night test periods, from the leaves on the different growth cycles. Aside from variations probably caused by branch and tree differences, the amounts of water absorbed from the leaves, which range from two to twenty months in age, are fairly similar under comparable conditions. Averaging the results as indicated in the figure it is found that of the water absorbed from the leaves during the day 15 percent came from the dorsal side and 85 percent from the ventral side, while of that absorbed during the night 38 percent came from the dorsal side and 62 percent came from the ventral side.

A comparison of the diurnal rates of loss from the leaves on all of the cycles shows the following:

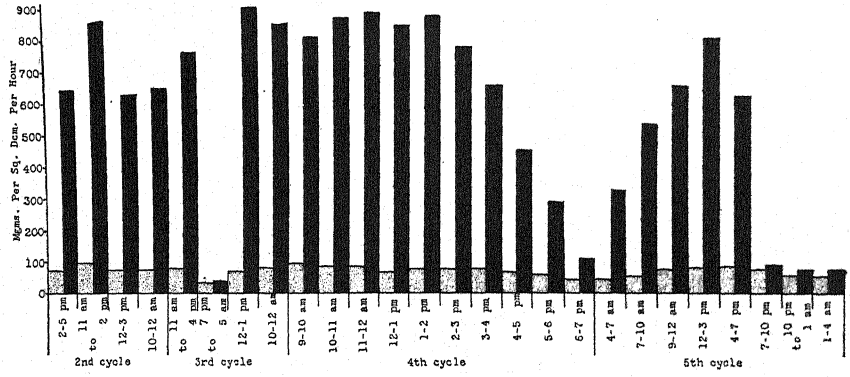
Loss from dorsal side—During the day, 71 percent
During the night, 29 percent
Loss from ventral side—During the day, 85 percent
During the night, 15 percent

In these computations daytime included the interval between 6 A.M. and 4:30 P.M., and nighttime the interval between 7 P.M. and 4:30 A.M.

Data similar to those given in text figure 2 for orange leaves are given in text figure 3 for lemon leaves. Here again the same four cycles of growth are represented, the fifth cycle being the oldest. The age of the leaves on the different cycles was approximately the same as that of the orange leaves. The dates on which the tests were made ranged from July 21 to October 24, 1930. In the tests on the fourth cycle the same lot of leaves was used during each test period, from 9 A.M. to 7 P.M. On the second, third, and fifth cycles a different lot of leaves was used in each test, and no two tests were made on the same branch, and seldom on the same tree.

The results recorded in text figure 3 show not only the relative amounts of water absorbed from the dorsal and ventral sides of the lemon leaves but also the relative amounts absorbed during the day and night test periods from the leaves on the different growth cycles. Allowing for the variations caused by branch and tree differences, the amounts of water given off by the lemon leaves at a given time of the day or night are not greatly different. The amount given off by the ventral side of the leaves during the day is always far in excess of that given off by the ventral side at night, and also

far in excess of that given off by the dorsal side during the day. During the night the amount given off by the ventral side does not greatly exceed that given off by the dorsal side. Averaging the results as indicated in the figure it is found that of the water absorbed from the leaves during the day 9



TEXT FIG. 3. Comparative rates of water loss from Eureka lemon leaves, on different cycles of growth, during different portions of the day and night, and from the dorsal and ventral surfaces of the leaves. Dotted columns denote the loss from the dorsal surface, solid columns the loss from the ventral surface.

percent came from the dorsal side and 91 percent from the ventral side; while of that absorbed during the night 40 percent came from the dorsal side and 60 percent came from the ventral side.

A comparison of the diurnal rates of loss from the leaves shows the following:

Loss from dorsal side—During the day, 65 percent
During the night, 35 percent
Loss from ventral side—During the day, 93 percent
During the night, 7 percent

In these computations daytime included the interval between 7 A.M. and 7 P.M., and nighttime the interval between 6 P.M. and 5 A.M.

A series of tests was made to determine the comparative resistance of Eureka lemon and navel and Valencia orange leaves of the most recent growth cycle to the loss of water. Another object of these tests was to make a similar determination of the comparative resistance of the young leaves near the tip and the older leaves near the base of the growth cycle. The number of leaves on each branch tested and the relative position of each leaf on the branch is indicated in table 2, the leaves being numbered basipetally.

The results given in table 2 show that the dorsal sides of the three sets of young leaves were very much alike in their resistance to the loss of water. There was a greater variation in the amounts of water lost from the ventral

TABLE 2. *Comparative Amounts of Water Absorbed by Calcium Chlorid from the Dorsal and Ventral Sides of Eureka Lemon, and of Navel and Valencia Orange Leaves* *

Test Period	Leaf Number, from Apex of Branch	Milligrams per Square Decimeter per Hour		Weather Conditions (Average for Each Test Period)		
		Dorsal	Ventral	Temperature, Deg. Fahr.	Relative Humidity, Percent	Wind Velocity, Miles per Hour
Eureka lemon; 32 leaves on branch; leaf No. 6 about 2/3 grown						
Nov. 24, 11–12 A.M.	6, 7, 8, 9	60	342	70	21	12
	25, 26, 27, 28	68	1,111	70	21	12
Navel orange; 29 leaves on branch; leaf No. 3 about 7/8 grown						
Nov. 26, 11–12 A.M.	3, 4, 5,	60	547	74	34	6
	23, 24, 25	68	983	74	34	6
Valencia orange; 22 leaves on branch; leaf No. 2 about 7/8 grown						
Dec. 4, 11–12 A.M.	2, 3, 4	68	479	70	35	3
	18, 19, 20	76	667	70	35	3

* The leaves in each case were located in two groups, one near the apex and the other near the base of the most recent growth cycle.

sides of the three groups of leaves. The navel orange leaves lost 34 percent and the Eureka lemon leaves lost 27 percent more than was lost from the Valencia orange leaves. When we compare the amount of water lost from the group of young leaves with that from the older leaves on the same branch we find that in each case the older leaves lost the most. This is true for both the dorsal and the ventral sides. The most marked difference in the amounts lost occurred in the case of the ventral side of the lemon leaves where the losses were 342 and 1,111 mgms. per sq. dcm. per hour, respectively, for the young and the older leaves. On any one of the three branches the difference between the ages of the youngest and the oldest leaf tested was probably not over four to six weeks.

The fifth series of tests was made to determine more directly the effects of light and darkness on stomatal movement. For this purpose Eureka lemon leaves were chosen. The leaves were on the fourth growth cycle from the tip of the branch, and were approximately fifteen months old. The tests were made in the middle of October, but the autumn growth cycle had not yet started to develop on these branches. In the first test the shells were attached to the leaves and the baskets of calcium chlorid were left in them for two hours, from 10 A.M. to 12 A.M. At 12 A.M. baskets of fresh calcium chlorid were inserted and the entire apparatus at once covered with two thicknesses of heavy dark cloth. The apparatus was left in this condi-

tion for two hours, from 12 A.M. to 2 P.M. The next day the test was repeated but in this case the leaves were darkened during the first half instead of the last half of the period. The entire period, as on the previous day, extended from 10 A.M. to 2 P.M. During the entire test period on each day the apparatus was shaded with cheesecloth, but in spite of this the leaves while covered with the dark cloth were subjected to a temperature that was two degrees higher than when they were not covered. The temperature inside the dark cloth on both October 15 and October 16 was 70° F.

TABLE 3. *The Stomatal Response of Fourth-Cycle Eureka Lemon Leaves as Indicated by Their Comparative Rates of Water Loss when in Darkness and when in Bright, Indirect Sunlight* *

Test Condition	Test Period	Milligrams per Square Decimeter per Hour		Weather Conditions (Average for Each Test Period)		
		Dorsal	Ventral	Temperature, Deg. Fahr.	Relative Humidity, Percent	Wind Velocity, Miles per Hour
In light.....	Oct. 15, 10–12 A.M.	86	752	65	49	2.5
In dark.....	Oct. 15, 12–2 P.M.	52	227	70	43	4.0
In dark.....	Oct. 16, 10–12 A.M.	43	252	70	54	3.0
In light.....	Oct. 16, 12–2 P.M.	69	684	78	37	4.5

* The leaves were approximately 15 months old, and the same leaves were used for all four tests.

The results given in table 3 indicate that the stomata on these leaves were noticeably sensitive to the effects of light and darkness. While in the dark on October 15 the dorsal side of the leaves gave off only 60 percent and the ventral side only 30 percent as much as they did in the light. Under similar conditions on October 16 the dorsal side of the leaves gave off only 62 percent and the ventral side only 37 percent as much as they did in the light.

Tests similar to those described thus far were made on leaves of the grapefruit and the sweet lemon, but the results are not recorded here because they would not add anything new to the results already given. The rates in which they lost water under the varying conditions agreed very closely with those of the Eureka lemon.

Another series of tests was conducted to determine the comparative rates of loss of water from the leaves of the first, second, and third cycles of growth on all of the five different varieties of citrus mentioned in the introduction. Here again, as in all cases already mentioned, the "first cycle" refers to the one at the distal end of the branch, hence the one most recently produced.

The results of the tests just mentioned showed that the leaves on the

second cycle of all varieties lost water more readily than those on either the first or third cycles. The losses from the leaves of the first and third cycles were about the same, but the losses from the leaves of the second cycles exceeded those from the first and third by approximately 45 percent in the case of the grapefruit and two lemon varieties, and 20 percent in the case of the two orange varieties.

In order to determine what effect the cutting of the branch might have on stomatal action the following tests were made. The shells were attached to six first-cycle leaves and the baskets of calcium chlorid inserted in the usual manner and left for a period of two hours, from 10 A.M. to 12 A.M. At 12 A.M. fresh calcium chlorid was inserted and immediately thereafter the lower end of the branch was cut under water where it was allowed to remain. At 2 P.M. fresh calcium chlorid was again inserted and allowed to remain until 4 P.M. This arrangement gave three two-hour periods, one before the branch was severed and two afterward. A similar series of tests was made also on leaves of the second and third cycles of growth. It was not difficult to make a test of this sort for it is not an uncommon thing for one or more of the lower branches of the citrus tree to curve downward at an angle of approximately 45° near its distal end. From near the end of this branch a secondary branch may grow upward in nearly a vertical position. Such vertical or nearly vertical branches as these were used in the tests, and it was an easy matter to sever the lower end of such branches under water without disturbing the set-up of the apparatus or the position of the leaves. The tests were made during the latter part of October.

The results of the tests on the leaves of the severed branches showed that the severing had a marked effect on the amounts of water lost from both the dorsal and ventral sides of the leaves, especially from those on the first cycle. The following percentages indicate the amounts of water lost by the leaves during each of the two periods after the branches were severed, as compared with the amounts lost during the period before severing, 10 A.M. to 12 A.M.

	12 A.M. to 2 P.M.	2 P.M. to 4 P.M.
First-cycle leaves—Dorsal	74	48
Ventral	20	4
Second-cycle leaves—Dorsal	56	38
Ventral	43	7
Third-cycle leaves—Dorsal	69	37
Ventral	93	24

Discussion

As stated in the first part of this paper, the method used in this investigation is subject to certain objections. The writer feels, however, that for the special purposes for which it was used it has given satisfactory results. It should be borne in mind that although portions of the dorsal and ventral sides of the leaves were exposed to dry calcium chlorid in a closed chamber for given periods of time, such exposures were probably less desiccating than

those to which the leaves were normally exposed during many of the summer and autumn days. Under the usual daytime conditions the air movements rapidly dissipate the inverted cones of water vapor which tend to form externally around each stoma pore. Within the closed chamber, during a test, the air was not in motion and the rate at which the vapor cones were dispelled depended upon the rate at which the calcium chlorid was able to absorb the moisture given off by the leaf. Under such conditions there would be a decreasing gradient of moisture outward from the leaf surface to the calcium chlorid. In these tests the distance from the leaf surface to the nearest side of the calcium chlorid baskets was approximately 2 cm.

It is felt that this method is more accurate than the cobalt chlorid method. The latter was tried in earlier tests, but was found to be unsatisfactory because (*a*) the end point was too indefinite, especially when the rate of water loss from the leaf was slow and several minutes elapsed before a reading could be taken; (*b*) the glass plate which held the test paper on the leaf permitted diffusion either toward or away from the paper; (*c*) if not held in position, the weight of the clip and glass plate or plates, if both sides are being tested, caused an abnormal twisting of the petiole; (*d*) the stomata on the citrus leaf are not evenly distributed, even within a given area, and the use of such a small piece of test paper naturally subjected the results to a certain amount of error because of this uneven distribution; and (*e*) the portion of the leaf covered by the test paper was subjected to almost complete darkness.

The results given for the fourth cycle in text figure 3 indicate that the conditions under which the tests were made did not materially influence the amount of water that normally would have been given off by these leaves. In this case the same set of leaves was tested hourly from 9 A.M. to 7 P.M. If the calcium chlorid had caused an abnormal decrease in the water content of the leaf or had caused the stomata to close or partially close to an unusual extent, these conditions would have been shown by the results. The tests indicated in table 3 and several other tests of this kind gave similar results. It should be stated, however, that in some cases the conditions of the test appeared to cause the stomata to close partially. The reason why this should happen in some cases and not in others is not clear. It does not seem improbable that the water content of the leaf at the beginning of the test may have been a factor. Some leaves would show this response much more noticeably than the adjacent or nearby leaves of the same growth cycle. In order to avoid these possible sources of error a new set of leaves was used for each test in the majority of cases.

The experimental results shown in this paper indicate that Coit and Hodgson (4) and Reed and Bartholomew (9) were in error in concluding that 40 to 50 percent of the water transpired by citrus leaves escapes through the cutinized epidermis of the dorsal surface of the leaf which contains no stomata, and that Coit and Hodgson were also in error in concluding that most of the stomata on mature citrus leaves have become permanently

closed and cannot function. Coit and Hodgson do not describe their method for determining cuticular transpiration, but the error of Reed and Bartholomew was apparently made by detaching the leaves before making the tests. The detaching of the leaves caused the stomata to close, thus making the comparative amounts of loss from the dorsal and ventral sides similar to those which occur during the night. The results of the present investigation which are partially given in tables 1, 2, and 3 and in text figures 2 and 3 show that during the day a comparatively small proportion of the water of transpiration escaped through the dorsal surface of the leaves. The results also show that the stomata, even on leaves that were at least twenty months old, were comparatively very efficient in regulating the loss of water from the leaves.

That the stomata are not permanently closed, or nearly so, on mature citrus leaves, but are plastic and responsive to external and internal influences, is not only indicated in the preceding, but is also indicated in the results given in table 3. It is shown here that only about one-third as much water was lost from the ventral side of the leaves during the periods of darkness as during the similar periods of diffuse sunlight. Eureka lemon leaves were used in this test, but Valencia orange leaves gave similar results. The results with the orange leaves showed just a little less contrast which would indicate that their stomata are slightly, though not much less, plastic than those of the lemon.

Further and perhaps more direct evidence that the stomata on mature citrus leaves open and close was obtained during the making of some tests which were preliminary to the present investigation. During this preliminary work the porometer method was tested as a possible means of measuring the loss of water from citrus leaves, but the method was discarded because it was not considered to be suitable for the desired purposes. During these preliminary tests, however, it was found that when the porometer was fastened to a third-cycle leaf on a lemon tree, it took the water column 75 seconds to drop from the 15 to the 10 cm. mark. The leaf petiole was then severed from the branch and at the end of 2 hours it took the water column 4 minutes and 35 seconds to drop the same distance. Since the rate at which the water column dropped depended upon the amount of air that passed through the pores of the stomata, it seems evident that the stomata on this leaf must have materially closed during the two-hour interval. Other tests gave similar results.

The stomata, as nearly as could be determined by this method, appear to begin to open about dawn, reach their maximum width about the middle of the forenoon or a little later, begin to close about the middle of the afternoon, and close rapidly as darkness approaches. These findings confirm those of Oppenheim (8) who based his conclusions on the rates of transpiration from the ventral side of mature leaves of *Citrus bigaradia* and *C. limetta*. Judging by the comparative amounts of water given off by the leaves at

different times during the day he concluded that the stomata began to open soon after sun-up, reached a maximum width about 10 A.M., and began to close about 4 P.M.

Bartholomew's (1) measurements of the diurnal contraction and expansion of lemon fruits lend further evidence as to the validity of the preceding conclusions as to the times at which citrus stomata open and close. He found that there was a relation between the time at which the stomata opened and closed and the time at which the fruits began to contract and expand. As would be expected there was a certain amount of lag between the opening and closing of the stomata and the contracting and expanding of the fruits, but nevertheless a direct connection.

It is impossible to state exactly what proportion of the water that was absorbed from the ventral surface of the leaves passed through the stomata and what proportions passed through the epidermis of the surrounding cells. Some idea of the comparative amounts may be obtained by comparing the rates of loss from the dorsal and ventral sides during the night when the stomata were closed. By reference to table 2 and to figures 2 and 3 it may be seen that always during the night less water was given off from the dorsal than from the ventral side of the leaves. This may be explained in at least two different ways: either more water actually passed through the epidermal cells which surround the stomata or else the stomata were not completely closed during the entire test period and part of the water escaped through them. It seems that both may have been responsible. Computing from the data in figures 2 and 3 it was found that during the night the Valencia orange leaves lost 1.7 and the Eureka lemon leaves lost 1.5 times as much water from the ventral side as from the dorsal. The epidermal layer of the dorsal side of the citrus leaf, which contains no stomata, is much more heavily cutinized than that of the ventral side.

According to the results obtained by Kamp (7), the differences between the amounts of dorsal and ventral transpiration of the leaves of many of our more or less common woody plants are less than those of citrus leaves. For example, he found that *Acer syriacum* lost only 2 times, *Coffea arabica* 4 times, *Rhamnus alnifolius* 4.3 times, *Quercus sessiliflora* var. *Darwinii* 3.7 times, and *Quercus rubra* 3.8 times as much water through the ventral side, which contained the stomata, as through the dorsal side, in which there were no stomata. Several other plants showed similar proportions while again others showed a much higher comparative rate of loss through the ventral side, the highest being *Laurus nobilis* which lost 45 times as much water through the ventral side as through the dorsal side. The average for the twenty plants which Kamp tested (see his table 7) showed that during the day 9.87 times as much water was lost through the ventral as through the dorsal side. This is almost exactly the same proportion as that lost by the ventral side of the Eureka lemon leaves during the day, the average for these leaves being 9.9. The loss from the ventral side of the Valencia

orange leaves during the day was somewhat less, being 6.3 times that from the dorsal side. This is exactly the same figure as that given by Kamp for *Quercus ilex*, the holly oak.

The total amounts of water lost from the grapefruit and Eureka and sweet lemon leaves were greater than those lost from the leaves of either the Valencia or naval orange. This was found to be true for both young and mature leaves. One of the explanations for this probably rests in the fact that the grapefruit and lemon leaves have more stomata per unit area than those of the oranges. Hirano (6) has found that there is considerable variation in the number of stomata on the leaves of many different species and varieties of citrus. He found, for example, that for every 100 stomata on the mature, average-sized leaf of the Lisbon lemon there were 82 on the Marsh grapefruit, 78 on the Eureka lemon, 63 on the navel orange, and 61 on the Valencia orange. While this shows that the average leaves of the grapefruit and the lemons have more stomata than those of the oranges, yet the fact that the Valencia orange leaves have approximately 80 percent as many stomata as those of the Eureka lemon but lost only about 64 percent as much water indicates that some other factor or factors may have been concerned. What some of these other factors may have been has been extensively discussed in other papers on leaf transpiration so they will not be mentioned here, except as they concern directly the results of this investigation.

Concerning the loss of water from citrus leaves it is of interest to note that the amount lost through the dorsal side of the leaf, in which there are no stomata, is also governed to a certain extent by light and darkness, temperature, and probably other factors. This is perhaps most plainly seen in table 3 where it is shown that the loss of water from the dorsal side of the leaves during the two hours of darkness was only about 60 percent of what it was during the two hours of light.

In making this investigation sun leaves were always chosen upon which to make the tests. This was done because Reed and Hirano (10) in working with citrus, Kamp (7) in working with other woody plants, and others who have worked on the factors governing transpiration rates have found considerable differences to exist between sun and shade leaves. For example, Reed and Hirano found that there were less stomata per unit area on citrus leaves grown in the shade than on those grown in sunlight, and Kamp found that the shade leaves transpired less water than those in sunlight. However, it was found in this investigation that although care was taken to choose sun leaves yet there was often a marked difference in the amounts of water lost by adjacent leaves, by adjacent groups of leaves on the same branch, and by groups of leaves on different branches. These differences could be explained only partially by the differences in the ages of the leaves. Another factor which may have been important is that suggested by Reed and Hirano, namely, that leaves produced while there is an abundance of available moisture have more stomata per unit area than those produced while available moisture is less abundant.

The preliminary work of Reed and Bartholomew (9) indicated that young citrus leaves give off less water than the mature ones. In general this has been confirmed by the results of the present investigation. These results show that the youngest leaves on the most recent growth cycle gave off less water than those near the base of the same cycle (table 2). They also show that all of the leaves on the most recent growth cycle gave off less water than those on the next older cycle, or what is termed in this paper the second cycle. What appear to be exceptions to this have been found with orange leaves in cases where only a very few leaves were produced on the youngest growth cycle and where the tests were made after these leaves had become almost mature.

The tests to determine the comparative rates of loss of water from the leaves on the different growth cycles showed that the leaves on the second cycle lost the most while the losses from the first, third, fourth, and fifth cycles were about equal. This conclusion is not drawn from the data presented in figures 2 and 3 but from data obtained by testing successively the leaves borne on the different cycles of growth all on the same branch. These results confirm those of Boeuf and Genet (3) who reported in 1906 that young orange leaves transpire less water than those which have just matured. However, they do not confirm their finding that in this respect the orange leaves differ from the other species of citrus. In the present investigation the young leaves of all five species of citrus tested lost less water than the newly-matured leaves.

Just why the severing of the lemon branches caused such a diminution in the amounts of water given off by the leaves is not evident. The results are presented here because the manner of making the test is to a certain extent analogous to the potometer method of testing transpiration, namely, the severing of branches, or whole plants, and allowing the cut ends to remain immersed in water while the test is being made. The results show that the response was more rapid and that the diminution was much more marked in the case of the leaves of the first cycle than in those of the third cycle. Of course, any substance or tissue alteration that would impede the flow of water in the vessels would cause a decrease in the quantity of water lost from the leaves. It is well known that if citrus tissues are wounded they readily form gum, but it does not seem probable that enough gum to clog the vessels could have been formed in such a short time. The very marked decrease in the amounts of water lost from the ventral sides of the leaves indicates that the closing or partial closing of the stomata was at least partly responsible for the decrease in the amounts of water lost. It should be noted, however, that there is also a decrease in the amounts of water lost from the dorsal sides of the leaves in which there are no stomata. From the results obtained in these particular tests it would seem that the potometer method may not always be reliable.

The temperature, relative humidity, and wind velocity that existed

during the test periods concerned are recorded in table 2 along with the other data. A record of these three weather conditions and for total hours of sunshine was kept for every test period during the entire investigation. However, except in table 3, such data have not been included with the other data because it is felt that under the conditions of the tests such external conditions were not of sufficient importance to make it advisable to record them. The records show, of course, that these external conditions did influence to a certain extent the actual amount of water lost by the leaves. The objects in most of the tests were to make comparisons, such as determining the comparative amounts of water given off by the dorsal and ventral sides of the leaves, the amounts of water given off by leaves on different growth cycles, and whether or not the stomata really continue to function after the leaves have become mature. In such cases the weather conditions were not of major importance.

While interpreting the results of the tests as given in this paper it should be kept in mind that on citrus trees that are over eight to ten years old the majority of the leaves are not over two cycles old, and that probably 90 percent of them are not over three cycles old. This is especially true of the leaves on orange trees. Trees under five or six years of age, such as were used in these tests, may retain some of their leaves until they are at least six cycles old. Since citrus leaves on a given cycle are considered to be mature by the time the succeeding cycle begins to develop and since the data in tables 1 and 3 and in figures 2 and 3 show that the stomata on leaves even on the fourth and fifth cycles of growth efficiently functioned in controlling the loss of water from the leaves, it seems safe to conclude that the stomata on these mature leaves had not become permanently closed. The results presented also indicate that the major portion of the water transpired by the leaves escaped through the stomata and not through the cutinized epidermis.

Summary

The results of the investigation concerning the loss of water from citrus leaves and some of the factors which govern this loss, as found to apply to the leaves of the first to fifth growth cycles of the Eureka and sweet lemon, the grapefruit, and the Valencia and navel oranges, may be summarized as follows:

1. Citrus leaves vary considerably in their rates of transpiration.

2. During the day 85 to 95 percent of the water lost by mature citrus leaves came from the ventral surfaces which contained the stomata.

3. During the night the loss of water from the ventral surface of mature leaves averaged only 1.5 to 1.7 times that from the dorsal surface.

4. The stomata on mature citrus leaves are able to respond to changes in external conditions, aside from the usual diurnal changes. This was very plainly shown by the fact that when subjected to total darkness for a two-hour period during the middle of the day, the amount of water lost by the

leaves was only approximately one-third of what it was during an immediately preceding or following two-hour period in which they were exposed to bright, indirect sunlight.

5. The light green, immature citrus leaves transpired less water per unit area than the mature ones.

6. The leaves near the apex of an immature growth cycle transpired less water per unit area than those near the base of the same cycle.

7. Leaves on the first, second, and third cycles of growth lost noticeably less water after the branches to which they were attached had been severed from the tree under water, and allowed to stand in water, than before they had been severed. This was especially true of the immature leaves on the first cycle.

8. Second-cycle leaves lost water more rapidly than those of the first, third, fourth, and fifth cycles.

9. The stomata on mature orange leaves appeared to be a little less responsive to environmental changes than those in the mature leaves of the lemons and the grapefruit. The mature leaves of the former also lost a little less water than those of the latter.

10. As indicated in a general way by these tests, the stomata on citrus leaves began to open about dawn or soon after, reached their maximum width soon after the middle of the forenoon, began to close about the middle of the afternoon, and closed fairly rapidly as darkness approached.

The preceding summarized results do not confirm those of previous workers which indicated that from 40 to 50 percent of the water transpired by the mature citrus leaves passes through the cutinized dorsal epidermis in which there are no stomata, and that the stomata on the mature leaves do not function, but become permanently closed.

CITRUS EXPERIMENT STATION,
RIVERSIDE, CALIFORNIA

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FLORAL DEVELOPMENT IN *DAUCUS CAROTA*

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Historical

The first investigations of the development of the inflorescence and flowers of Umbelliferae were made by Payer (6) in 1853. He studied species of *Heracleum*, *Carum*, *Aegopodium*, *Anethum*, *Phellandrium*, and others, and concluded that the involucral rays arise as isolated primordia, each ray usually subtending an umbellet. Younger umbellets in the center of the umbel, however, do not always possess a subtending ray. Payer apparently believed that the sepals, petals, stamens, and carpels arise in acropetal succession, the five members of the sepal whorl alternating with the five of the petal whorl, and these in turn with the five stamens. He says that two sepals are anterior, two lateral, and one posterior. In carrot we find that a line which separates the two carpels divides the flower in such a way that there are on the side (anterior) next to the axis of the umbellet, two sepals, three petals, and two stamens, whereas on the opposite side (posterior) there are three sepals, two petals, and three stamens. This is probably the condition referred to by Payer in the preceding statement. He stated that the two lateral sepals of the three on the posterior side appear last, but that it is impossible to determine with certainty whether the other three develop simultaneously or successively. Payer said that the petals all arise at the same time, as do also the stamens. His discussion of the development of the carpels is not detailed but in its general features agrees with findings of subsequent investigators.

Jochmann (3) concluded that the usual arrangement of flowers in the umbellets is verticillate, but that sometimes there is a spiral arrangement. This is at variance with Sieler's (7) emphatic statement that the primordia both of umbellets in the umbel and of flowers in the umbellet always arise in a spiral manner. Sieler claimed, moreover, that Jochmann is erroneous in contending that there are sometimes divergences greater than 2/5. Sieler maintained that the prevailing divergence among the Umbelliferae is 2/5.

Jochmann, as did Payer, regarded the calyx as the first floral whorl to appear. With this view also Sieler differed, claiming that the primordial calyx of Payer and Jochmann is in reality the cycle of stamen primordia.

Sieler concerned himself chiefly with the order of development of the parts of the inflorescence and the order of appearance of the individual members of the cycles of the flower. As stated above, he stressed the point

that in the umbel, as in the umbellet, the consecutive members are inserted in a spiral arrangement with a 2/5 divergence. As regards the origin of umbellet primordia, Sieler described two cases, represented by *Peucedanum parisiense* DC and by *P. cervaria* Lap. The umbellet primordia in the first case appear with a regular 2/5 divergence, which means that the sixth umbellet primordium stands directly over the first, the seventh over the second, etc. The second case is more involved. This, he stated, is similar to *P. parisiense*, except that the spiral runs in the opposite direction and that the divergence between successive series of five members each is $\frac{2-1/2}{5}$, rather than 2/5. He meant to say that whereas the divergence between members of a single series of 5 is 2/5, or 144°, the divergence between the 5th and 6th umbellet primordia, last and first respectively of two consecutive series, is $\frac{2-1/2}{5}$, or 108°. For example, the members of the second series will stand over no member of the first series, but primordia of the third series will stand directly over primordia of the first, in such a way that primordium number 11 stands directly over primordium number 3; likewise the second and fourth series will align vertically.

With the observations we have made with fresh material of *Daucus Carota* L., employing high power dissecting binoculars and very favorable lighting, we have been unable either to credit or discredit Sieler's conclusions. We find it difficult to understand how he could ascertain the divergence between successive members of a series of primordia, much less a divergence of $\frac{2-1/2}{5}$, such as described above. Moreover, our paraffin series have been of little help to us in this particular.

Sieler described three types of floral development. In the first two of these the primordia have a spiral arrangement with a divergence of 2/5. In the third type, the spiral arrangement is not so apparent. In the first type, which includes among others *Pastinaca sativa*, the stamen primordia are the first to appear, followed by the corolla, then the calyx (if any), and last the carpels. In the second type, to which belongs *Daucus Carota*, stamens and sepals develop simultaneously and prior to the other primordia. In the third type, which is rare and represented by *Foeniculum officinale*, stamen and petal primordia appear alternately, the development starting at one point and proceeding around both sides of the flower. More specifically, the anterior petal is the first to appear, then the two stamens immediately adjacent, then the two middle petals, followed by the stamens adjacent to them, and finally the two posterior petals and the single posterior stamen. The sepal primordia arise in irregular succession after petal and stamen primordia have been formed, these to be followed by carpel primordia. Usually in this type the sepals do not develop.

Concurring with Payer and Jochmann, Sieler showed that the carpels are the last to develop. Sieler added that the two carpels develop at different times, another observation which we find difficult to verify in *Daucus Carota*.

Bartsch (1) investigated rather carefully nine different species of Umbelliferae, including *Daucus Carota*. He gave also a very satisfactory literature review of previous work. He stated that Treviranus (8) erroneously assumed the presence of a calyx tube in the Umbelliferae. Bartsch pointed out that the calyx consists of five rudimentary teeth or that it may be entirely absent. He fully described the structure of the stylopodium, styles, and stigmas. The production of an inferior ovary was accounted for by the growth of a ring-like zone of the axis which raises all of the primordia except the carpels.

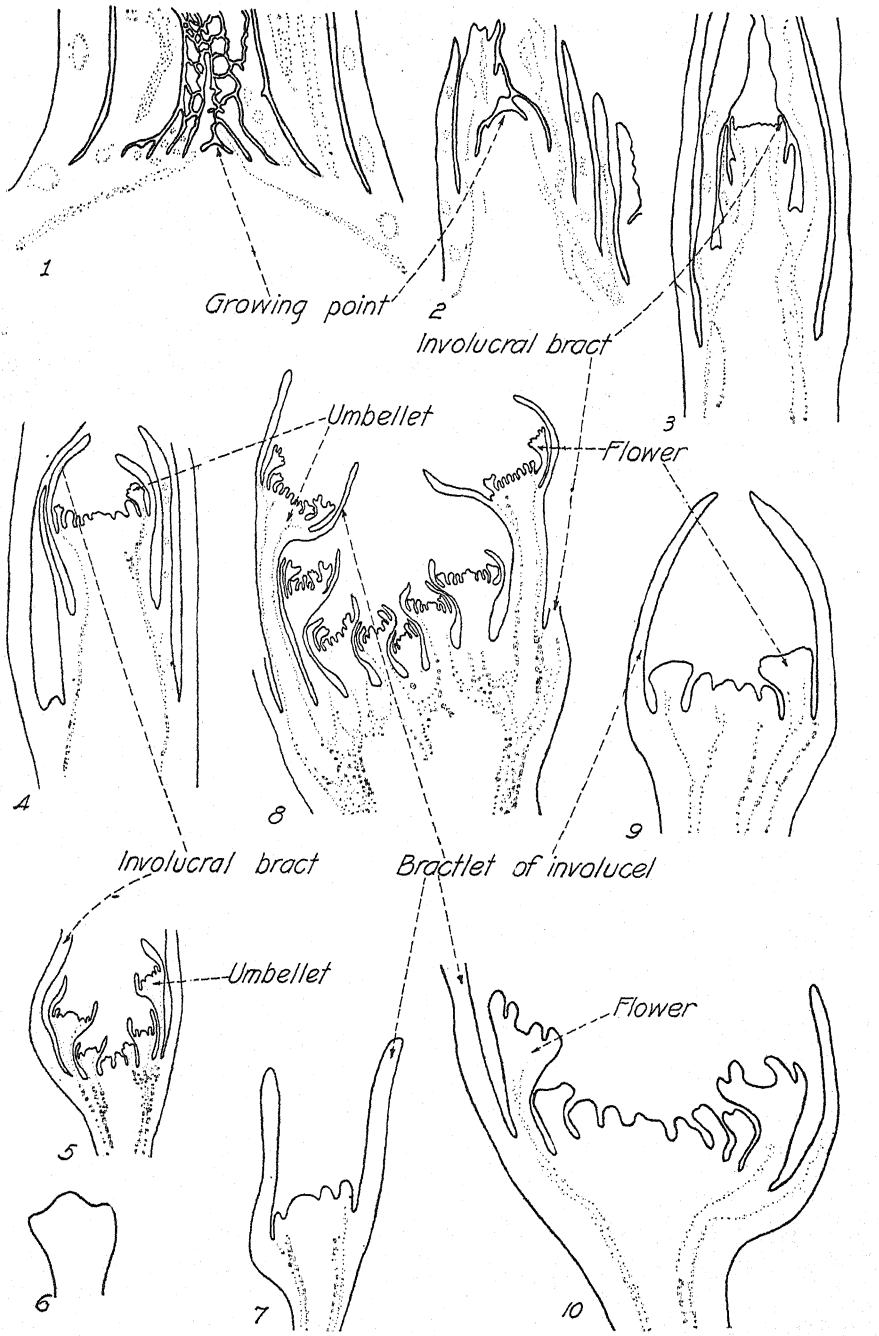
Martel (5) came to the rather unusual and apparently erroneous conclusion that the umbelliferous pistil consists of two whorls of five "phyllomes" each, in other words of ten carpels. He believed that the ovary consists of the stylopodium only, and that from this structure ovules arise, while the cavity in which the ovules are suspended is formed by the elongation of the receptacle.

Cammerloher (2) incorrectly interpreted Martel as stating that the gynoecium consists of four carpels, rather than ten. All of the workers except Martel held that the four ovules arise from the inturned margins of the two carpels.

Jurica (4), working with *Eryngium yuccaefolium*, described for this plant a regular acropetal succession of floral organs. Jurica incorrectly interpreted Sieler's findings, at least as they concern the floral type of development to which *Daucus Carota* belongs. In this species Sieler says that the succession is not acropetal, but that the stamen and sepal primordia appear simultaneously, and are followed by the corolla, as pointed out above. In *Eryngium yuccaefolium* Jurica found nothing unusual in the development of the carpels and ovules, except that in one entire head two normal ovules developed in each ovary cavity.

Development of Inflorescence and Flowers in Daucus carota

Roots set in late December (Davis, California) had within a month produced several leaves about 2 to 3 inches long (Pl. LII, *A*). At this stage of development the growing point of the stem was very slightly convex (text fig. 1). By the middle of February top growth (Pl. LII, *B*) had increased and the growing point had become more cone-shaped (text fig. 2). By March 15 there was a well-developed top (Pl. LII, *C*). At this time the flowering axis had become several inches long, and its apex had attained the stage of development shown in text figure 8. In the meantime, lateral buds on this main axis had arisen, their tips reaching stages of development shown in text figures 3 and 4. As the stem tip elongates its very apex tends to flatten, and around the periphery of this flattened apex the primordia of involucral bracts appear as small protuberances of undifferentiated tissue. As these increase in size they are followed centripetally by umbellet primordia; finally, the entire stem apex is covered with these primordia, in slightly different stages of development.

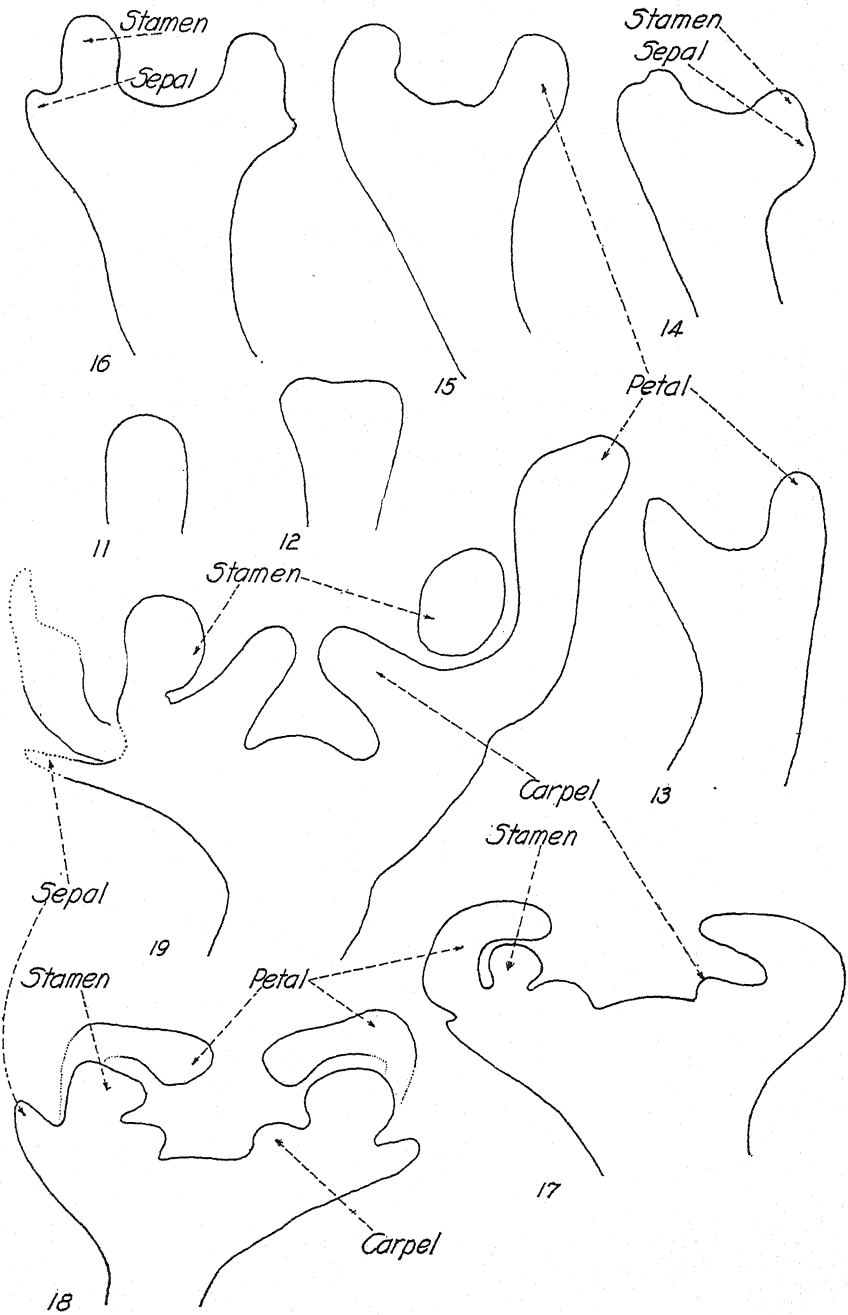


TEXT FIGS. 1–10. 1, longitudinal section through growing point of main axis when root is at stage of development shown in Plate LII, *A*; 2, same when top growth is as shown in Plate LII, *B*; 3, 4, 5 and 8, successive stages in the development of an umbel; 6, 7, 9 and 10, successive stages in the development of an umbellet. In 10 ...

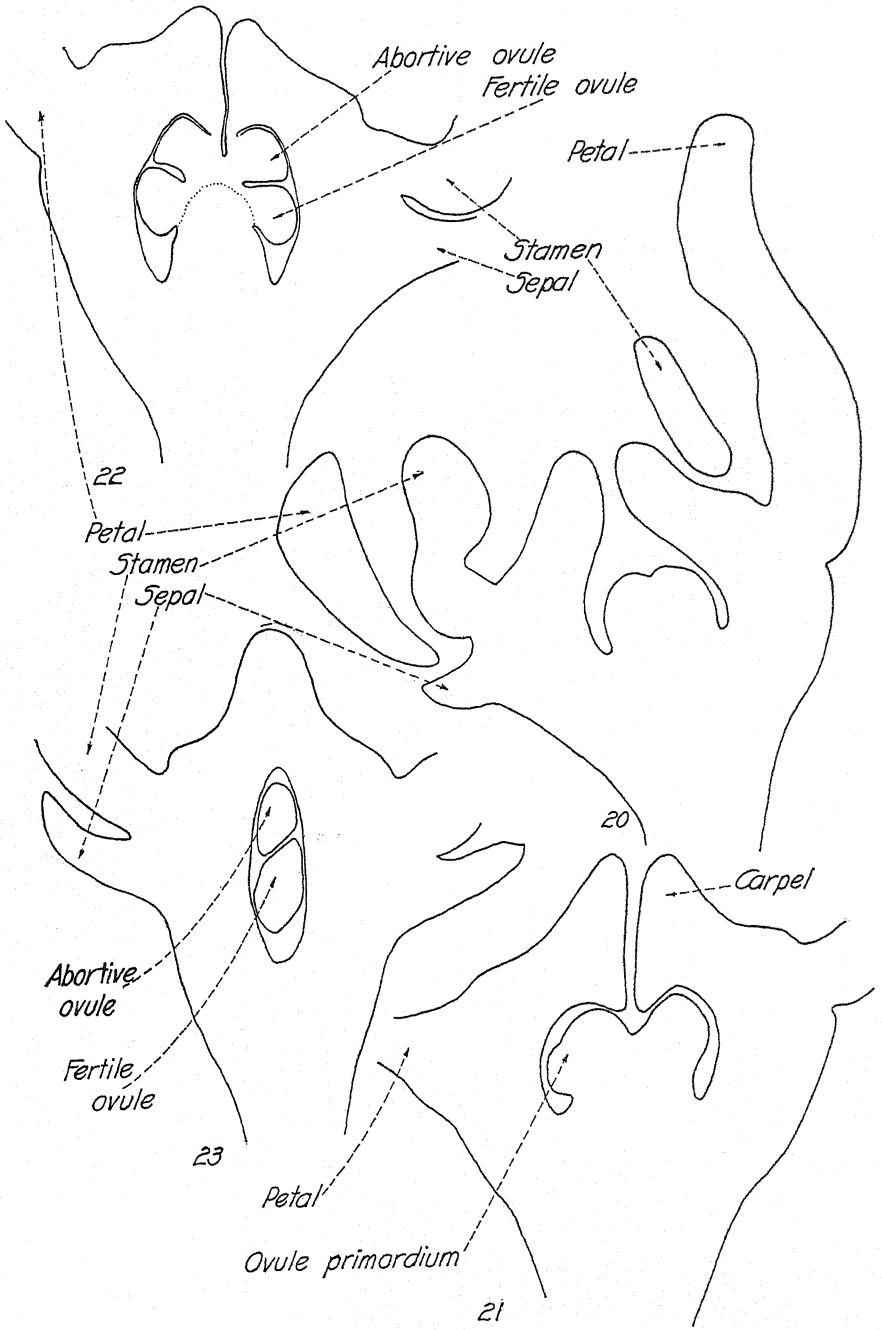
The development of the umbel is best understood by referring to text figures 3, 4, 5 and 8; and of the umbellet to figures 6, 7, 9 and 10. As early in the life of the plant as is illustrated in Plate LII, *C*, primordia of floral organs are in evidence (text fig. 10). Figures 11 to 19 are early stages in the development of the flower. The cone-shaped flower axis soon flattens out and broadens. Sepal, petal, and stamen primordia appear so nearly simultaneously that it is difficult to be certain of the order of development. The stamen primordia are immediately above those of the sepals. Petal primordia soon surpass in size sepal and stamen primordia. Carpel primordia are the last floral structures to develop. As pointed out early by Payer, the five petal primordia alternate on the one hand with the five sepal primordia and on the other with the five stamen primordia. As stated above, in the carrot we find that a line which separates the two carpels divides the flower in such a way that there are on the side (anterior) next to the axis of the umbellet, two sepals, three petals, and two stamens, whereas on the opposite (posterior) side there are three sepals, two petals, and three stamens.

Carpel development in the carrot is similar to that described very early by Bartsch who studied various Umbelliferae.

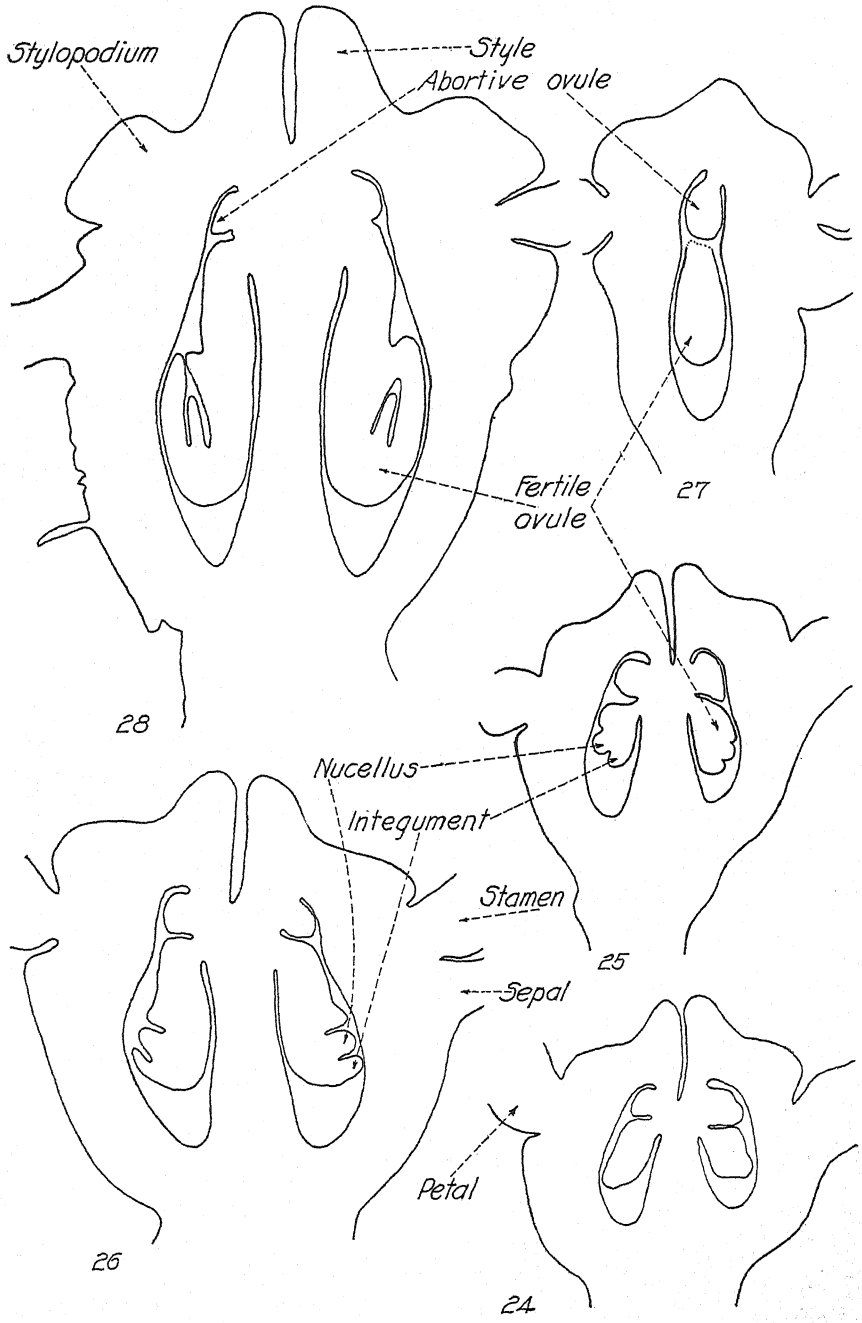
After the appearance of the primordia of sepals, petals, and stamens there is still left in the center of the flower a circular area of meristematic tissue which is nearly flat. This region is destined to give rise to two carpels, so oriented in the flower that a radius of the umbellet will bisect both carpels. First evidence of carpel formation is shown by a slight rise at two points on opposite sides of this circular area of meristematic tissue. Text figure 31 represents this entire area while *a* of this figure shows the positions of the carpels when first evident. These two protuberances are at first nearly circular in cross section (text fig. 32), but as they grow, more of the meristematic tissue lateral to them begins to elevate until the two regions of elevating tissue as viewed from above become crescent shaped (text fig. 33). Longitudinal views of these regions are shown in text figures 17–19. The arrows in text figure 31 show the directions in which the two areas of elevating tissue extend themselves. In this figure the high point of each carpel is at the center, *a*, and each carpel slopes from this point to its margins, *b*, which are still at the level of the rest of the surrounding meristematic tissue. These primordial carpels continue to elevate, and as they do so they extend laterally assuming first the appearance shown in text figure 34 and finally in figure 35. As a result of this development the cavity in the ovary is gradually divided into two locules, one in each carpel (text fig. 22). The partition which separates the two locules from each other results from the upgrowth of the carpel margins which are turned inward, meeting in the center. As seen in longitudinal sections (text figs. 19–22) of the flower, it appears to arise from the floor of the ovary and to grow up through the cavity, dividing it almost completely into two locules (text fig. 22). Since this partition is formed by the inturned margins of two carpels (text figs. 34–35), there should be some



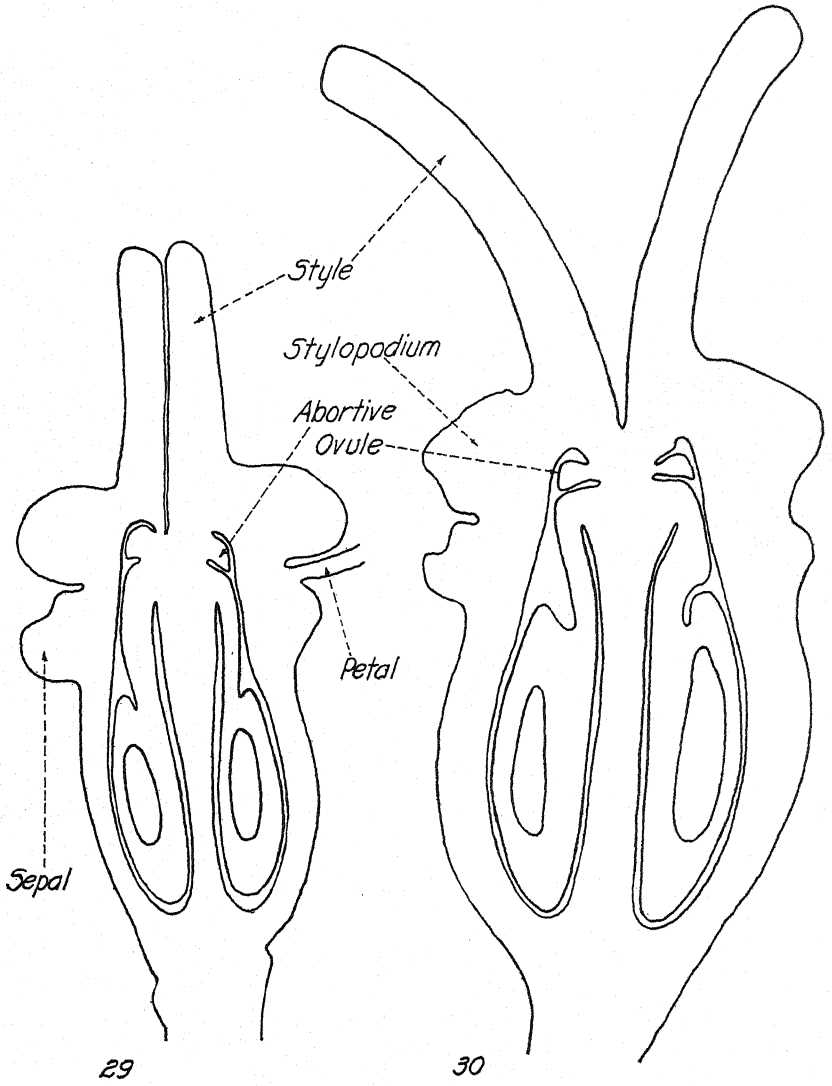
TEXT FIGS. 11–19. Successive early stages in the development of the flower. 13, section through petals on each side: 14, same flower as 13, cut through sepals and stamens. × 190.



TEXT FIGS. 20–23. Successive later stages in the development of the flower. 22 and 23 are different views of flowers of the same age, but older than 20 and 21. In 20, observe that the fused edges of the carpels form a partition across the base of the ovary, partly dividing the cavity into two locules. × 190.



TEXT FIGS. 24–28. Successive later stages in the development of the flower. 24, abortive ovules approaching their greatest size; 25, nucellus and the beginning of the single integument appear; when the ovary has reached this stage the megaspore mother cell is about ready for reduction division; 26, somewhat older than 25, four megaspores may be found in nucellus; 27, same as 26, but cut in a plane at right angles to that shown in 26; 28, ovule turned completely back on itself; embryo sac in



TEXT FIGS. 29 AND 30. Two late stages in floral development. 29, flower open at this stage, but styles not yet separated from each other; embryo sac contains eight nuclei, but fertilization not effected; stamens have fallen, but petals still remain on the flower. 30, at about time of fertilization; all of the petals have fallen and styles have spread apart. × 75.

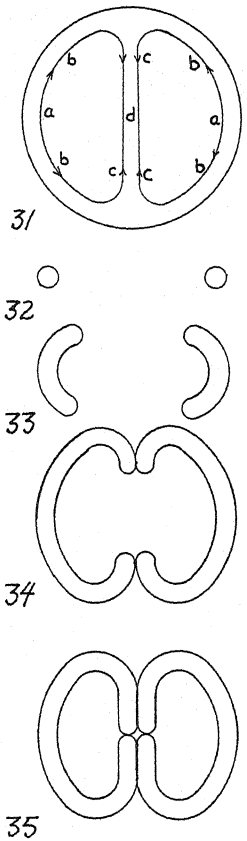
indication of its double structure. This evidence is found in the notch-like depression shown at the top of the partition in longitudinal sections of young flowers (text figs. 20–21). The ease with which the two mericarps separate from each other at maturity further supports this point of view.

From the inturned margins of each carpel two ovule primordia develop,

one slightly above the other. As a rule only one ovule, the lower, in each locule becomes functional (text figs. 21–30).

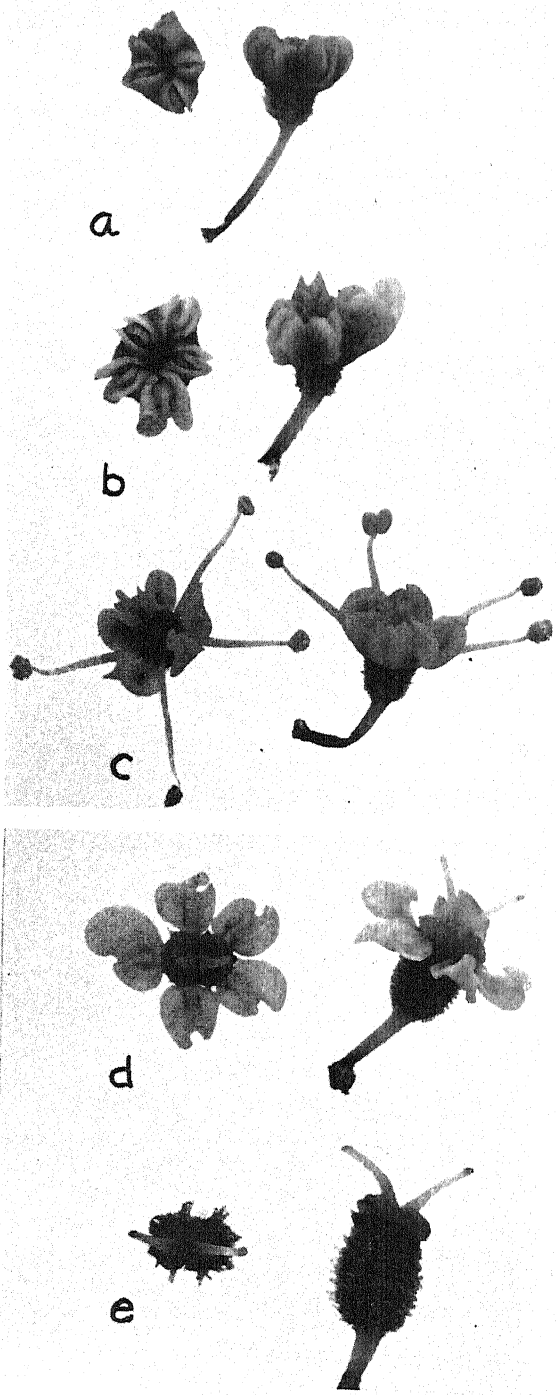
The stylopodium of carrot is well developed (text figs. 28, 29, 30). The material under study threw no new light on the morphological nature of this structure. However, it is quite evident that Martel's interpretation that ovules arise from the stylopodium is not correct.

From the foregoing discussion it is seen that the primordia of floral structures are differentiated very early during the second season's growth.



TEXT FIGS. 31–35. Diagrams illustrating stages in development of carpels. See text for discussion.

At the time the top growth consists of a few leaves, several inches in length, the primordia of umbel and umbellets are well differentiated. In the material examined, within three months after the roots were set, there was developed a mass of foliage, hidden within which was a very short stem bearing the primordia not only of umbels and umbellets, but of floral organs as well. To the casual observer, this axis would not be noticed. The elongation of



TEXT FIG. 36. Stages in development of carrot flower. × 6.

the axis is rapid, as is also the development of all floral structures. Prior to the opening of the flower and to style elongation, the abortive ovule attains its greatest size, the functional ovule turns completely back on itself, and the embryo sac reaches about the 8-nucleate stage (text figs. 24–28 and 36, *a* and *b*). The flowers open irregularly. The filaments are inrolled in the unopened flower, but have a considerable length. At anthesis, the petals separate slightly, the anthers are released, and the filaments straighten. Straightening of the filaments is not simultaneous, however. Dehiscence of the anthers ensues very shortly after the filaments become straight. As soon as pollen is shed, the entire stamen falls. In any one flower it may happen that, before the last filament has straightened out, certain others have shed their pollen and fallen. Style elongation and flower opening occur approximately at the same time. Text figure 29 represents a stage of development somewhere between that shown in figure 36 *c* and *d*. At this stage the petals are still intact, the stamens have fallen, but the stigmatic surfaces are not receptive. There is further elongation of the styles (text fig. 30), during which they separate, and the stigmas become receptive. At this stage all the petals and stamens have fallen and fertilization has taken place. Text figure 30 represents a stage that is slightly younger than figure 36.

That fertilization acts as a stimulus to petal fall is shown by the fact that clipping of the stigmas before pollination results in the retention of the petals.

Summary

1. Umbel primordia in carrot roots set in late December at Davis, California, are already differentiated by the first of March. At this time the plant has produced a large growth of leaves and stem elongation has just begun.

2. Sepal, petal, and stamen primordia are formed nearly simultaneously. These are followed by carpel primordia.

3. Opening of the flowers is irregular.

4. Dehiscence of anthers and falling of stamens occur before the stigmas become receptive.

5. Fertilization takes place after the styles have separated from each other and just before petal fall.

These studies are a part of more comprehensive investigations of the factors which influence carrot seed production.

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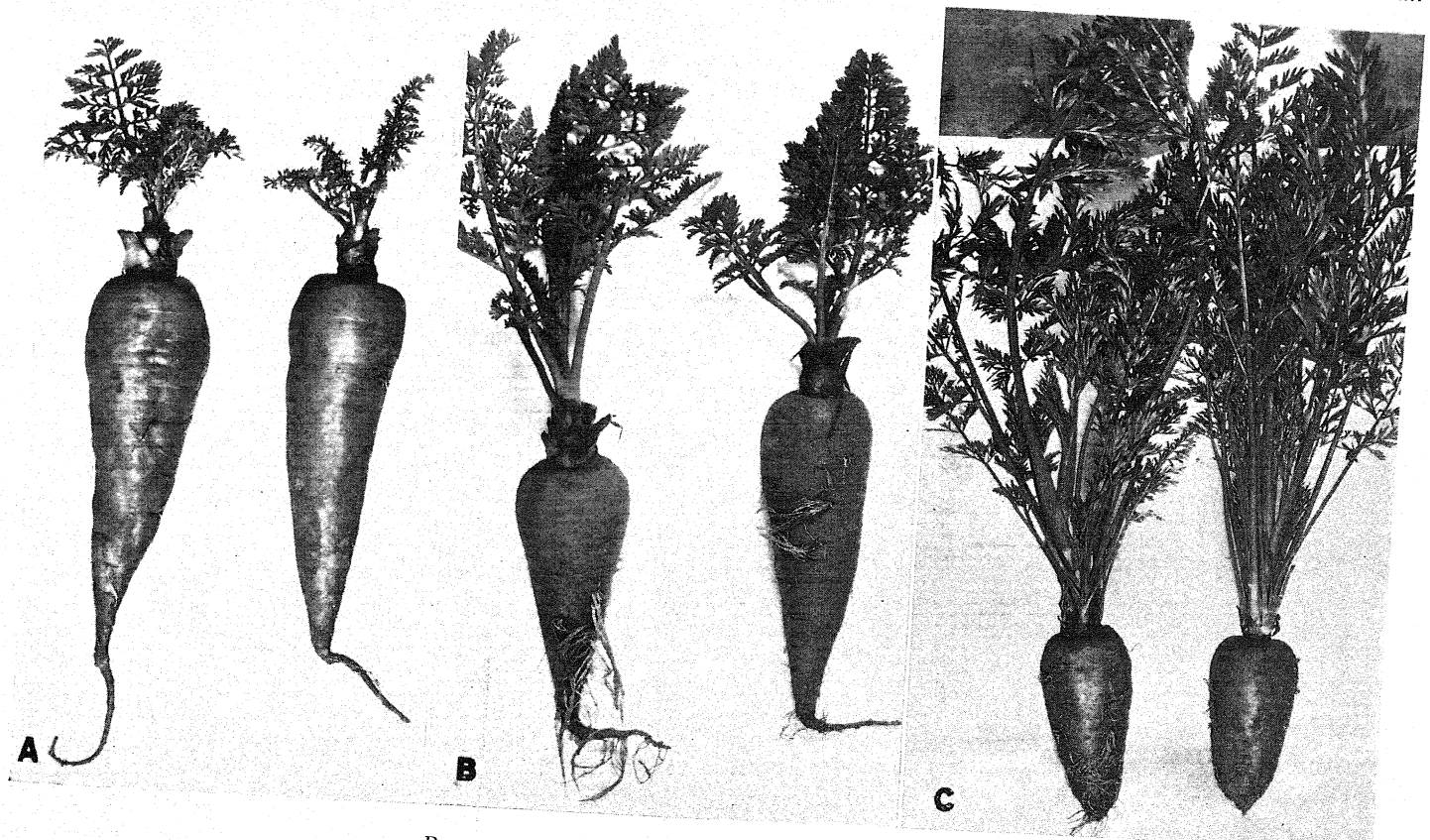
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DESCRIPTION OF PLATE LII

Three stages of development of top growth made by carrot roots set in late December Davis, California). *A*, late January; *B*, middle of February; *C*, middle of March. *A* and *B*, $\times \frac{1}{2}$; *C*, $\times \frac{1}{4}$.



BORTHWICK, PHILLIPS AND ROBBINS: DAUCUS FLOWER

STUDIES IN *SAXIFRAGA* II. TERATOLOGICAL PHENOMENA IN CERTAIN NORTH AMERICAN SPECIES OF *SAXIFRAGA*

A. M. JOHNSON

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A considerable number of the North American species of *Saxifraga* studied by the writer showed a marked frequency of occurrence of abnormalities of various sorts in the flowers. Most of these species belong in the sections Hydatica, Dermasea, and Micranthes (1, 2, 3), these being the sections containing the most species and the ones of which the most material was available for study. It is likely that other sections of the genus may show similar abnormalities.

In the absence of careful field and cytological investigations the causes of these abnormalities are not easy to explain. Hybridity may account for many of them, if not for all, and this may at the same time in a measure explain the instability and polymorphism of the various complex aggregates in which clear-cut specific lines are so difficult to draw; and it is in these puzzling species complexes that so many of these abnormalities appeared, *e.g.*, the *Saxifraga saximontana*, *S. fragosa*, and *S. nidifica* complexes (3). In the herbarium material it was not possible to discover any indication of traumatic origin of any of these abnormalities, although the black condition of many of the aborted stamens might suggest such origin. That ecological factors otherwise may have anything to do with these phenomena seems hardly probable.

The most marked abnormalities and the species in which they occur are as follows:

Section HYDATICA

Saxifraga aestivalis F. & M. Great diversity in size and shape of petals and sepals. A tricarpellate gynoecium was found in one flower.

Saxifraga arguta D. Don. Great diversity in size and shape of petals and sepals, and considerable variation in the configuration of the apex and in the depth of the apical notch of the petals. Sepals entire to irregular and ciliolate on margins. Two aborted stamens were found in one case and in another all the petals were aborted. In both these cases the sepals were more or less aborted.

Saxifraga Nelsoniana D. Don. Here were found several cases in which the sepals were exceedingly irregular in shape, with entire and glandular ciliate to irregularly dentate margins. Flowers were observed with 2-carpellate, 3-carpellate, and 4-carpellate gynoecia.

Saxifraga unalaschensis Sternb. Diversity in size and shape of petals

and sepals appears to be quite general, with marked inconstancy in shape of the filaments, these ranging from subulate to subclavate. Carpels varied from two to four.

Saxifraga Lyallii Engl. Considerable diversity in size, shape, and marginal characters of sepals and petals. In many cases these organs were exceedingly irregular in outline. The petals were often notched at the apex. In some cases the filaments were usually broad. Carpels generally 2, but often 3, 4, or 5. Coalescence of the sepals occasional.

Section DERMASEA

Saxifraga aequidentata (Small) Johnson. Diversity in size and shape of petals and sepals. Filaments ranging from clavate to subulate, often very irregular and indefinite in shape. A 4-carpellate gynoecium present in a number of cases.

Saxifraga Allenii (Small) Johnson. Considerable diversity in size and shape of petals and sepals. Coalescence of filaments *inter se* and more or less with petals frequent.

Saxifraga laevicaulis Johns. Great diversity in size and shape of petals and sepals. A 3-tricarpellate gynoecium occasional.

Saxifraga microcarpa Johns. Fasciation of stamens noted. Carpels frequently 3.

Saxifraga occidentalis Watson. Suppression of a carpel and coalescence of sepals occasional. Entire flower abnormal in a number of cases.

Saxifraga petiolata Johns. Irregularities in size and shape of petals and sepals common. A 3-carpellate gynoecium occasional.

Saxifraga rufidula (Small) Johns. Considerable diversity in size and shape of petals and sepals, with a tendency of the petals to become lobed. Coalescence and forking of the filaments noted in several instances.

Saxifraga saximontana E. Nelson. Great diversity in size and shape of petals and sepals, with a tendency of the petals to become suborbicular and more or less deeply notched or cleft at the apex, suggesting coalescence. Coalescence and fasciation of the filaments appeared to be frequent. A number of cases were found in which the fasciations bore supernumerary anthers at the summit, below which were (apparently) normal anthers (Pl. LIII, fig. 14). Otherwise the filaments showed all gradations from the normally clavate to an irregular or distinctly subulate type.

Saxifraga tennesseensis Small. Here also there was found considerable diversity in the configuration of the sepals and petals. Besides 3-carpellate gynoecia occasional abortions of stigma and style were found.

Saxifraga virginiensis Michx. Here were extreme diversities in size and shape of sepals and petals, but no malformations of any sort were discovered in the numerous specimens examined.

Saxifraga idahoensis Piper. Similar extreme diversities in the configuration of the sepals and petals were found in this species, the petals showing a

tendency toward deep notching at the apex. In some cases the sepals were glandular ciliolate. A 3-carpellate gynoecium was found in several cases, the carpels showing both partial and more or less complete union.

Saxifraga franciscana (Small) Engler & Irmscher. Here, too, petals and sepals were extremely diverse in size and shape. One case was found of what appeared to be carpellody of a stamen—the filament being very much fasciated, with numerous small ovule-like bodies at the summit, the whole partly adnate to a deeply notched sepal (Pl. LIII, fig. 18).

Saxifraga rhomboidea Greene. All flowers examined of a large number of specimens referred to this species showed great diversity, often extreme, in size and shape both of petals and sepals. The sepals ranged from small, narrow, deltoid ones, 1.2 mm. long, to broad oblong ones, 2.7 mm. long, with entire to often very irregular margins, the larger ones supplied with 3 to 5 or 6 veins from the base. In a number of cases the sepals were more or less deeply cleft at the apex. Coalescence of petals was frequent, these forms being supplied with two principal veins, closely approximated, from the base. Several flowers showed malformations of the stamens.

Section TRICARPUM

Saxifraga texana Buckley. Here there was wide diversity in size and shape both of petals and sepals, frequently accompanied by coalescence, the larger organs being supplied with 3 to 6 principal veins from the base, and frequently deeply notched at the apex. A number of cases of malformations of the stamens were found. Gynoecia of 2, 3, and 4 carpels appeared to be of about the same relative frequency, but where 3 or 4 carpels were present, one of them was, as a rule, more or less reduced or rudimentary.

Section ARABISA

Saxifraga ferruginea Graham. Similar differences appeared in the sepals and petals of this species as in the foregoing species. Among the normally hastate or sagittate petals deviations to a lanceolate or oblong shape were common, various intergrading forms often present in the same flower. Not infrequently unsymmetrical forms occurred, or forms that were half lanceolate and half sagittate. Several cases of coalescence of petal with sepal were noted.

Section MICRANTHES

Saxifraga californica Greene. Considerable diversity in size and shape of petals and sepals. Other than one case of coalescence of a petal with a sepal no unusual features were found.

Saxifraga fragosa Suksdorf. Considerable diversity in size and shape of petals and sepals. Apical notching of the petals was common, in which cases the petals were rounded or obtuse at the apex. Irregularity in the corolla appeared in several cases, three of the petals being large and broad, the remaining two quite small and narrow. A similar irregularity appeared in

the calyx. Abortion and other malformations of the stamens were frequent, as were also petalody of the stamens and coalescence of filaments with petals. Partial staminody of a petal appeared in one flower. A few flowers showed 12 stamens and 6 petals, and some showed a 3-carpellate gynoecium. In these 3-carpellate gynoecia one of the carpels was but half the size of the other two.

Saxifraga montanensis Small. This species of doubtful status has been described as apetalous, but several specimens referred by the present writer to this species showed presence of petals. A solitary minute petal (1 mm. long) was found in a flower of a specimen collected by F. H. Burglehaus at Mammoth Hot Springs, Yellowstone National Park, altitude 6000 feet, and labeled "*Saxifraga integrifolia.*" Another flower of the same specimen bore a lanceolate petal, 2.5 mm. long. A specimen from the collection of F. V. Hayden ("Headwaters of the Missouri and Yellowstone Rivers." Missouri Botanical Garden, No. 84147), which may be referred to this species, similarly showed the occasional presence of small petals.

Saxifraga nidifica Greene. Petals and sepals extremely variable in all collections referred to this species by the present writer (3). Malformations and coalescence of petals with sepals were frequent. In collections referred to *S. plantaginea* Small and *S. columbiana* Piper the petals ranged in shape from linear or club-shaped to broadly spatulate or orbicular, these different forms often being present in the same flower. Some flowers possessed only 4 sepals—3 broad ones and 1 small and narrow. Frequent also were partial and complete petalody of the stamens, and there was a striking frequency of supernumerary carpels, although these were generally more or less rudimentary or variously malformed. Among these forms were flowers possessing as many as 6, 7, and 8 carpels. Tricarpellate gynoecia were more frequent. The supernumerary carpels were usually of curious shapes and of various sizes, in many cases apparently being modified stamens, in others being partly a modified stamen, and partly a modified petal (Pl. LIV, fig. 8). An extreme case was discovered in which no trace of a gynoecium was to be found, but in its place was a flat discoid surface over which were spread in broken masses numerous small, apparently aborted, ovules (Pl. LIV, fig. 11).

Saxifraga oregana Howell. A specimen from Cusick's collection (Missouri Botanical Garden, No. 84458) showed coalescence and fasciation of filaments, but the anthers were apparently normal. Petals and sepals were of diverse shapes and sizes.

Saxifraga sierrae (Coville) Small. Specimens referred to this species by the present writer (3) showed numerous malformations, involving all the floral organs and extending to the floral bracts. As broadly interpreted this species is apparently exceedingly polymorphic as regards vegetative characters, but no clear-cut distinctions could be found, either in floral, fruit, or seed characters to warrant segregations. Petals and sepals differed widely in size and shape, respectively, even in the same flower, with a

tendency to irregularity both in calyx and corolla. Coalescence of petals, of sepals, and of petal with sepal was common. Petals ranged in shape from elliptical to oblong or orbicular, often with very irregular outline, with margin entire on one side and dentate on the opposite side, and showing considerable inconstancy in the venation. There were several cases of petalody of the sepals. The androecium showed abortions of various sorts. In several flowers the inner set of stamens was considerably longer than the outer set, and there were several cases of petalody of the carpels. Tricarpellate gynoecia were frequent, as were cases of fusion of the carpels. In some flowers there was only one carpel present. Staminody of the carpels was found in one case.

Summary

Several, especially west-American, species of *Saxifraga* were found to show a rather frequent occurrence of malformations of the floral organs. In general these malformations were most frequent in those species complexes which are the most difficult taxonomically. Such teratological features as irregularities in shape, fasciation, and coalescence of the floral organs *inter se*, petalody of stamens and sepals, abortion of the stamens or of the anthers only, and of the carpels, were especially frequent. Among the more unusual features were a number of cases of open carpels suggesting phyllody of these organs, a few cases of supernumerary carpels, and one case of complete suppression of the carpels, leaving the more or less aborted ovules exposed on a fleshy discoid surface. While definite information is wanting the suggestion may be made that these phenomena may possibly be due to hybridization.

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DESCRIPTIONS OF PLATES

Plate LIII

Fig. 1. A flower of *Saxifraga fragosa* var. *leucandra* Suks. (Suksdorf's No. 4015), with abnormal stamen, petal, and sepal on right: *a*, *b*, *c*.

Fig. 2. Detail of abnormal organs in figure 1; *a*, anther; *f*, filament; *p*, petal; *s*, sepal.

Fig. 3. A tricarpellate gynoecium in *Saxifraga fragosa* var. *leucandra* Suks. (Suksdorf's No. 4015). The supernumerary carpel, *c*, partially aborted.

Fig. 4. Complete union of ovaries in gynoecium in *Saxifraga idahoensis* Piper. From Sandberg's collection, Missouri Botanical Garden No. 775342.

Fig. 5. Incomplete union of ovaries in gynoecium in *Saxifraga idahoensis* Piper. From same collection as in figure 4.

FIG. 6. Supernumerary carpel, *c*, in a flower of *Saxifraga nidifica* Greene, with its ventral side facing outward. From Sukdorf's No. 1728 (as "*S. bracteosa*").

FIG. 7. Floral bract fused with calyx-tube. From a flower of *Saxifraga sierrae* (Coville) Small. Baker's No. 4428 (as "*S. oregana* Howell").

FIG. 8. Forked filament from a flower of *Saxifraga tennesseensis* Small. Missouri Botanical Garden, No. 760519.

FIG. 9. A similarly forked filament from a flower of *Saxifraga rufidula* (Small) Engl. and Irmsch. From Macoun's collection, U. S. National Herbarium, No. 219591.

FIG. 10. Normal (right) and aborted (left) stamens from a flower of *Saxifraga saximontana* E. Nels. From Sheldon's No. 8536.

FIGS. 11, 12, 13, 14. Fused and fasciated stamens in flowers of *Saxifraga saximontana* E. Nels. From Sheldon's No. 8536. Right-hand fasciation in figure 14 shows supernumerary anthers at summit; *p*, petal; *s*, sepals.

FIGS. 15, 16. Normal and abnormal stamens in flowers of *Saxifraga texana* Buckl. Figure 15 from Ness's No. 1994; figure 16 from Palmer's No. 1647.

FIG. 17. Partly petaloid stamen from *Saxifraga microcarpa* Johns. From Elrod's No. 98, in the Herbarium of the University of Montana.

FIG. 18. Fasciation and carpellody of a stamen or stamens in a flower of *Saxifraga franciscana* (Small) Engl. and Irmsch. From Knowlton's No. 127 (as "*S. nivalis*").

FIG. 19. Side view of figure 18.

FIGS. 20, 21. Coalescence of stamens with petals in a flower of *Saxifraga Allenii* (Small) Engl. and Irmsch. From Allen's No. 242 (as "*S. nivalis* L.").

FIG. 22. Abnormal carpel from a flower of *Saxifraga nidifica* Greene. From Sandberg's collection, Lewiston, Idaho (as "*S. nivalis* L.").

PLATE LIV

FIG. 1. Fused and fasciated filaments and complete suppression of a carpel in a flower of *Saxifraga nidifica* Greene; *a*, aborted anther. From Hall and Chandler's No. 1675.

FIG. 2. Flower of *Saxifraga nidifica* Greene, from Sandberg's collection, Lewiston, Idaho (as "*S. nivalis* L.") Stamens 12, one (*s*) petaloid; anthers of aborted stamens shaded black; anthers of three of the other stamens prominently mucronate. At left (*p*) an abnormal petal. Both carpels partly aborted.

FIG. 3. Another flower from the same collection as figure 2. One carpel completely suppressed, the ventral side of the remaining carpel being shown, with the gland girdling it completely; *p*, a filament-like petal; *a*, an abnormal anther. Other organs removed for purposes of illustration.

FIG. 4. Partly coalesced and aborted carpels and partly aborted stamens in a flower of *Saxifraga nidifica* Greene, from the same collection as figure 2.

FIG. 5. A solitary carpel from a flower of *Saxifraga nidifica* Greene, the other carpel completely suppressed. From the same collection as figure 2.

FIG. 6. Aborted gynoecium from another flower of the same collection as figure 2; *f*, an abnormal filament; *g*, abnormal gland.

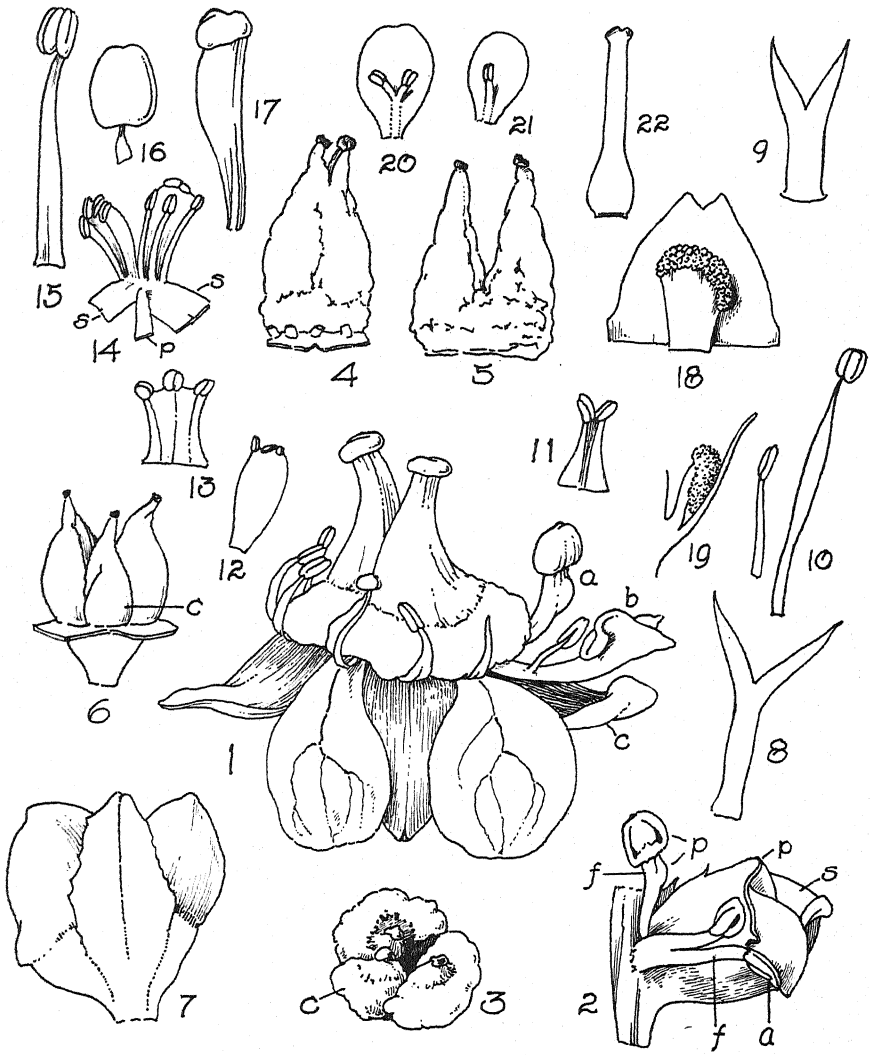
FIG. 7. Incomplete phyllody of a carpel in a flower of *Saxifraga nidifica*. Sandberg collection, Lewiston, Idaho (as "*S. nivalis* L."); *c*, a small supernumerary carpel.

FIG. 8. Phyllody of carpels and abortion of stamens and petal in a flower of *Saxifraga nidifica*, from the same collection as figure 7; *c*, a small supernumerary carpel; *p*, aborted petal; *s*, aborted stamens. Opposite the small aborted petal (*p*) is a stamen with an abnormal, acutely pointed anther.

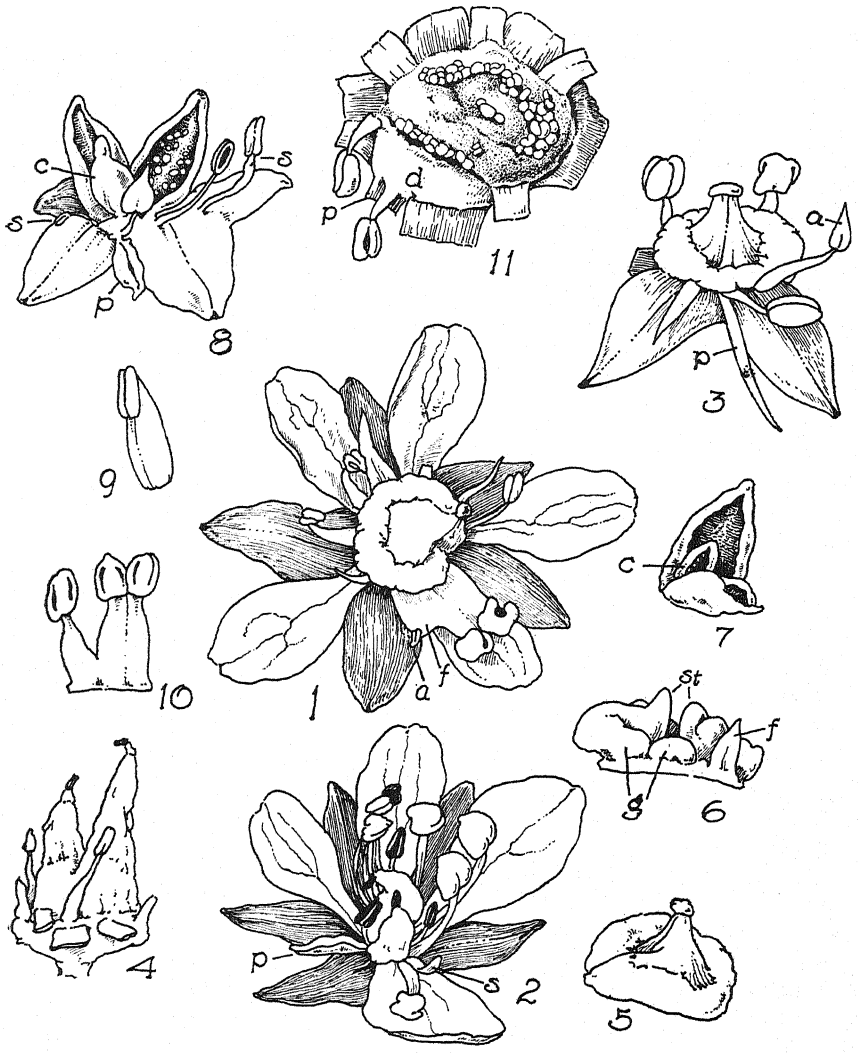
FIG. 9. Stamen with fasciated filament. From the same collection as figure 7.

FIG. 10. Fused and fasciated stamens from same collection as figure 7.

FIG. 11. Complete abortion of carpels in a flower of *Saxifraga nidifica* Greene. From Sandberg collection, Lewiston, Idaho (as "*S. nivalis* L."). The irregular masses of more or less aborted ovules are seen lying on the discoid surface of the malformation; *d*, a fleshy discoid outgrowth supporting a stamen and a petal (*p*).



JOHNSON: SAXIFRAGA



JOHNSON: SAXIFRAGA

INHERITANCE OF SMUT RESISTANCE IN HYBRIDS OF EARLY GOTHLAND AND MONARCH OATS [1]

GEORGE M. REED

(Received for publication September 22, 1931)

My strains of Early Gothland and Monarch oats differ in a very striking fashion in their behavior to the Missouri races of loose (*Ustilago avenae* (Pers.) Jens.) and covered (*U. levis* (K. & S.) Magn.) smuts. Early Gothland has consistently shown a high degree of susceptibility to the loose smut and, at the same time, it has been almost entirely resistant to the covered smut. The Monarch variety has reacted in exactly the opposite manner, proving to be extremely resistant to the loose smut, while showing complete susceptibility to the covered (1, 2, 3, 6, 10).

During the five years 1926–1930 various types of experiments have been carried out with these two varieties in which plants were inoculated with either the loose or the covered smut. Thirteen different experiments were made in which 279 plants of Early Gothland were inoculated with loose smut and of these 263 (94.2 percent) were infected; in ten of these experiments all the inoculated plants were smutted. In comparative experiments, 586 plants of this oat variety were inoculated with the covered smut and only 6 (about 1 percent) were infected. During the same years, 526 plants of Monarch oats were inoculated with loose smut in thirteen experiments, and only 6 (1.1 percent) were infected. On the other hand, 452 plants were inoculated with the covered smut, and 445 (98.4 percent) were infected; in eleven out of the thirteen experiments all of the plants were smutted.

The most obvious morphological difference between these two oat varieties is the color of the glumes or hulls of the ripe grain; in Early Gothland the glumes are white or pale yellowish, while in Monarch they are blackish brown. Early Gothland is a rather late maturing oat, with moderately large panicles, the branches of which droop slightly at the outer ends. The spikelets are usually two-grained, sometimes three-grained; the outer grains are rather long (18–22 mm.), slender, and long pointed. Awns are rather numerous on the spikelets of the panicles, but usually they are not fully developed nor twisted at the base.

Monarch is a somewhat earlier maturing oat. The stem and sheaths are dark green and quite glaucous at the time of heading. The panicles are rather wide-spreading, more or less lax, with the branches drooping from the middle outward. Awns are rather common in the panicle, but rarely

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ever have a twisted base. The spikelets are usually two-grained; the glumes are light brown at the base, becoming darker brown to almost black in the middle portion, and the elongated tips are whitish; the glumes also have a very distinct glaucous appearance. The grains are somewhat elongate, the outer one usually being 15 to 19 mm. in length, and long pointed.

Crosses between Early Gothland and Monarch

Hybrids between these two oat varieties furnish very favorable material for the study of the inheritance of smut resistance, since they offer such marked contrast in their reaction to the loose and covered smuts. Experiments have been carried out with the second, third, and fourth generations of the hybrids, and brief reports of some of these results have been published (8, 9).

In previous publications (4, 5) we have described our results on the inheritance of smut resistance in various oat hybrids. Extensive studies have been made with hybrids of Hull-less × Black Mesdag. In this cross, Hull-less is very susceptible to both the loose and covered smuts, while Black Mesdag is extremely resistant to both. The cross between Silvermine and Black Mesdag presents a similar situation, since Silvermine is quite susceptible to both loose and covered smut. A different condition is presented in crosses between Early Gothland and Victor, and Early Gothland and Hull-less. One of the parental varieties used in these crosses, namely, Early Gothland, is susceptible to loose smut but resistant to covered, while Victor and Hull-less are highly susceptible to both smuts. A similar type of combination is found in the cross between Hull-less and Monarch; in this case, Hull-less is susceptible to both smuts, while Monarch is susceptible only to the covered smut.

Three crosses between Early Gothland and Monarch were made in 1927 and in two of these (Hybrid 75-A and B) Early Gothland was used as the female parent, while in the other (Hybrid 76), Monarch was the female parent. In 1928 the F_1 plants were grown in the greenhouse. They resembled rather closely the Monarch variety. Relatively few of the spikelets had awns and, for the most part, they were non-geniculate; only occasionally did they have a twisted base. The outer grains of the spikelets were slender, brownish tan at the base, darker in the middle portion, with the tips whitish. The characteristic glaucous appearance of the Monarch grain was also evident.

In 1929 the second generation of these hybrids was grown. A set of 50 plants belonging to two of the hybrids was inoculated with the loose smut and grown to maturity in the greenhouse. Another set of 86 plants, belonging to all three of the hybrids, was inoculated with the covered smut, and some of these were grown in the greenhouse and others in the field. In addition to these two sets of inoculated plants, 60 uninoculated F_2 plants were grown to maturity in the field or in the greenhouse. Thus, there were

three different groups of the F_2 plants, depending upon whether they were inoculated with the loose smut, inoculated with the covered smut, or uninoculated.

The methods which we have generally used for inoculation of oat seed with the smuts were employed in the experiments. The inoculated seed was germinated in sand, with a moisture content of about 20 percent, and at a temperature of about 20° C. in the constant-temperature tank. When the seedlings were about a week old they were transplanted either to the pots in the greenhouse or direct to the field.

Data Obtained with the F_2 Generation

The data obtained with the second generation plants are recorded in table 1. Fifty plants of Hybrids 75-A and 76 were inoculated with the loose smut and of these 9 (18 percent) were infected. At the same time, 86 plants belonging to all three hybrids were inoculated with the covered smut and 9 (10.4 percent) were infected. Half of the plants of Hybrid 75-A and all of the plants of Hybrid 76 were grown in the field, the others maturing under greenhouse conditions.

TABLE 1. *Results with Inoculated F_2 Plants of Crosses between Early Gothland and Monarch*

	Inoculated with *Ustilago Avenae*			Inoculated with *Ustilago levis*		
	No. Plants	No. Inf.	Percent Inf.	No. Plants	No. Inf.	Percent Inf.
Hybrid No. 75-A	20	4	20.0	38	4	10.2
Hybrid No. 75-B	—	—	—	20	2	10.0
Hybrid No. 76	30	5	16.6	28	3	10.7
	50	9	18.0	86	9	10.4
Early Gothland	19	19	100.0	52	0	0
Monarch	19	0	0	44	44	100.0

It is interesting to note that a higher percentage of infection of the second generation plants was obtained with the loose smut and, further, that there was not a great deal of variation in the results secured with the plants of the two different hybrids. Further, all three hybrids gave approximately the same percentage of infection with the covered smut, the average being 10.4 percent.

Along with these F_2 plants there were grown inoculated plants of the parental varieties and the results obtained were in harmony with their usual behavior. Early Gothland gave 100 percent infection with the loose smut and entirely negative results with the covered. Monarch behaved in exactly the opposite manner, giving negative results with the loose smut, while being completely susceptible to the covered.

Data Obtained with the F_3 Generation

As noted above, the F_2 plants of the three hybrids were divided into three groups, as follows: (1) inoculated with the loose smut; (2) inoculated with the covered smut; (3) grown from uninoculated seed. For convenience, the plants of the first group were numbered between 1 and 100, those of the second group were numbered between 101 and 200, and those of the third group were numbered beginning with 201.

Two sets of third generation plants were grown from most of the F_2 plants, one set being inoculated with loose smut and the other with the covered. Some of these matured in the greenhouse and, in these cases, usually 15 seeds were used in each experiment. The remaining progenies were grown in the field and generally 25 seeds of each were inoculated.

The detailed data for the F_3 generation are presented in tables 2, 3, and 4. In each table the progenies are arranged on the basis of the treatment of the F_2 plants—whether inoculated with the loose smut, with the covered smut, or uninoculated. Further, under each group the progenies are separated on the basis of their behavior. All the F_3 progenies in which no infected plants occurred are grouped first; these are followed by the progenies which had less than 50 percent of the individuals infected; finally, the progenies of high susceptibility are recorded. The data on the F_3 progenies for both

TABLE 2. *Results with F_3 Progenies of Hybrid No. 75-A, Early Gothland × Monarch*

F_2 Plant No.	F_3 Plants Inoculated with *Ustilago Avenae*			F_2 Plant No.	F_3 Plants Inoculated with *Ustilago levis*		
	No. Plants	No. Inf.	Percent Inf.		No. Plants	No. Inf.	Percent Inf.
F_3 progenies descended from F_2 plants inoculated with *Ustilago Avenae*							
2*	15	0	0	4	15	0	0
5*	15	0	0	9*	15	0	0
6*	15	0	0	11*	17	0	0
7*	14	0	0				
8	15	0	0				
10*	15	0	0				
1*	15	2	13.3	1*	15	3	20.0
3*	15	1	6.6	3*	15	2	13.3
4	15	4	26.6	6*	14	2	14.2
9*	15	7	46.6	7*	15	5	33.3
				8	15	2	13.3
				10*	15	1	6.6
				12*	24	2	8.3
				13*	19	8	42.1
				14*	24	6	25.0
				2*	14	9	64.2
				5*	14	7	50.0

* The F_2 plants marked with an asterisk had dark glumes, the others had light glumes.

TABLE 2.—*Continued.*

F_2 Plant No.	F_3 Plants Inoculated with *Ustilago Avenae*			F_2 Plant No.	F_3 Plants Inoculated with *Ustilago levis*		
	No. Plants	No. Inf.	Percent Inf.		No. Plants	No. Inf.	Percent Inf.
F_3 progenies descended from F_2 plants inoculated with *Ustilago levis*							
107*	15	0	0	104*	15	0	0
109	15	0	0	105	15	0	0
				107*	14	0	0
				111*	22	0	0
				112*	23	0	0
				114	23	0	0
				117*	24	0	0
101*	15	3	20.0	101*	15	3	20.0
102*	15	7	46.6	102*	15	1	6.6
103*	15	4	26.6	103*	15	2	13.3
104*	15	2	13.3	108*	15	1	6.6
105	15	4	26.6	109	15	5	33.3
106*	15	1	6.6	110*	13	1	7.6
108*	15	1	6.6	113*	24	1	4.1
110*	13	1	7.6	115*	19	1	5.2
				118*	20	3	15.0
				106*	15	9	60.0
F_3 progenies descended from uninoculated F_2 plants							
202	14	0	0	203*	14	0	0
203*	13	0	0	210*	14	0	0
207	15	0	0	211*	21	0	0
210*	14	0	0	213*	23	0	0
				214*	20	0	0
201*	13	6	46.1	201*	13	2	15.3
204	14	1	7.1	202	15	1	6.6
206*	15	1	6.6	204	15	1	6.6
209*	15	1	6.6	205*	11	1	9.0
				207	15	2	13.3
				208*	14	4	28.5
				209*	14	5	35.7
				215*	25	4	16.0
				216*	23	3	13.0
				217*	18	5	27.7
205*	12	8	66.6	206*	15	15	100.0
208*	14	8	57.1	212	22	14	63.6

* The F_2 plants marked with an asterisk had dark glumes, the others had light glumes.

TABLE 3. *Results with F_3 Progenies of Hybrid No. 75-B, Early Gothland × Monarch*

F_2 Plant No.	F_3 Plants Inoculated with *Ustilago Avenae*			F_2 Plant No.	F_3 Plants Inoculated with *Ustilago levis*		
	No. Plants	No. Inf.	Percent Inf.		No. Plants	No. Inf.	Percent Inf.
F_3 progenies descended from F_2 plants inoculated with *Ustilago levis*							
104*	13	0	0	101	15	0	0
105*	15	0	0	103*	15	0	0
108*	15	0	0	104*	15	0	0
				105*	15	0	0
				106*	15	0	0
				108*	15	0	0
				109*	15	0	0
				110*	14	0	0
				116	18	0	0
				118*	21	0	0
101	15	6	40.0	102	15	3	20.0
102	15	2	13.3	107*	15	4	26.6
103*	15	6	40.0	111*	24	1	4.1
106*	15	5	33.3	112*	21	3	14.2
107*	15	1	6.6	113*	23	2	8.6
110*	15	3	20.0	114	24	4	16.6
				115	21	2	9.5
109*	15	15	100.0				
F_3 progenies descended from uninoculated F_2 plants							
208*	14	0	0	204*	14	0	0
209	14	0	0	207*	15	0	0
				211*	25	0	0
				215	24	0	0
				217*	23	0	0
				218*	23	0	0
201*	12	2	16.6	201*	12	5	41.6
202*	15	3	20.0	203*	15	2	13.3
203*	15	7	46.6	205	14	1	7.1
204*	15	6	40.0	206	14	4	28.5
205	15	3	20.0	209	14	2	14.2
206	13	5	38.4	216*	21	3	14.2
210	14	1	7.1	220*	22	9	40.9
207*	14	14	100.0	202*	15	15	100.0
				208*	15	10	66.6
				210	15	15	100.0
				212	23	23	100.0
				213	23	23	100.0
				214	25	25	100.0

* The F_2 plants marked with an asterisk had dark glumes, the others had light glumes.

TABLE 4. *Results with F_3 Progenies of Hybrid No. 76, Monarch × Early Gothland*

F_2 Plant No.	F_3 Plants Inoculated with *Ustilago Avenae*			F_2 Plant No.	F_3 Plants Inoculated with *Ustilago levis*		
	No. Plants	No. Inf.	Percent Inf.		No. Plants	No. Inf.	Percent Inf.
F_3 progenies descended from F_2 plants inoculated with *Ustilago Avenae*							
4	15	0	0	4	15	0	0
9*	15	0	0	5*	14	0	0
10*	15	0	0	6*	14	0	0
				10*	15	0	0
				15	24	0	0
1*	15	3	20.0	1*	14	5	35.7
2	15	1	6.6	2	15	4	26.6
5*	15	6	40.0	3*	15	3	20.0
6*	15	3	20.0	7*	15	2	13.3
7*	15	1	6.6	8*	15	2	13.3
8*	15	3	20.0	11	24	2	8.3
				13*	22	2	9.0
				14*	24	4	16.6
3*	15	8	53.3	9*	15	15	100.0
				12	23	14	60.8
				16*	24	24	100.0
F_3 progenies descended from F_2 plants inoculated with *Ustilago levis*							
106	13	0	0	101*	14	0	0
				102*	14	0	0
				108*	13	0	0
				110	15	0	0
				111*	11	0	0
				112*	20	0	0
101*	13	5	38.4	103*	14	3	21.4
102*	15	1	6.6	105*	14	1	7.1
103*	14	3	21.4	106	12	5	41.6
105*	14	2	14.2	107*	14	2	14.2
107*	9	2	22.2	109	15	4	26.6
108*	15	6	40.0				
109	15	4	26.6				
110	14	3	21.4				
111*	11	5	45.4				
				113	21	12	57.1
				114*	24	18	75.0

* The F_2 plants marked with an asterisk had dark glumes, the others had light glumes.

TABLE 4.—*Continued.*

F_2 Plant No.	F_3 Plants Inoculated with *Ustilago Avenae*			F_2 Plant No.	F_3 Plants Inoculated with *Ustilago levis*		
	No. Plants	No. Inf.	Percent Inf.		No. Plants	No. Inf.	Percent Inf.
F_3 progenies descended from uninoculated F_2 plants							
207*	15	0	0	202*	15	0	0
209*	13	0	0	210*	15	0	0
				218*	24	0	0
202*	15	7	46.6	201*	13	6	46.1
203*	15	1	6.6	203*	13	3	23.0
204*	15	2	13.3	204*	15	1	6.6
205*	13	1	7.6	205*	15	2	13.3
206*	15	4	26.6	206*	14	4	28.5
208*	14	2	14.2	207*	15	3	20.0
210*	15	3	20.0	208*	14	2	14.2
				209*	15	3	20.0
				211*	22	1	4.5
				212*	25	6	24.0
				213	22	2	9.0
				215*	23	3	13.0
				216*	22	1	4.5
				217	23	9	39.1
				219*	23	5	21.7
201*	15	14	93.3	214	25	25	100.0

* The F_2 plants marked with an asterisk had dark glumes, the others had light glumes.

smuts are presented side by side in these tables, but the order is necessarily not the same on account of their different reaction to the two smuts.

Results Obtained with Ustilago avenae

The data secured with the loose smut, as recorded in tables 2, 3, and 4, are summarized in table 5. As shown in this table, 20 progenies descended

TABLE 5. *Summary of Results with the F_3 Progenies of Hybrids 75-A, 75-B and 76, Inoculated with Ustilago Avenae*

Hybrid	F_2 Plants Inoculated with *Ustilago Avenae*			F_2 Plants Inoculated with *Ustilago levis*			F_2 Plants Not Inoculated		
	Resist.	Seg.	Sus.	Resist.	Seg.	Sus.	Resist.	Seg.	Sus.
75-A	6	4	0	2	8	0	4	4	2
75-B	—	—	—	3	6	1	2	7	1
76	3	6	1	1	9	0	2	7	1
	9	10	1	6	23	1	8	18	4

NOTE. Resistant progenies contained no infected individuals, segregating progenies had less than 50 percent of the individuals infected, and susceptible progenies gave 50 to 100 percent infection.

from F_2 plants inoculated with the loose smut were grown, 9 of which proved to be entirely resistant, 10 gave a percentage of infection varying from 6.6 to 46.6, and one (Hybrid 76-F_3-3) gave 53.3 percent smutted plants. Thirty F_3 progenies descended from F_2 plants inoculated with covered smut were grown, and 6 of these were entirely resistant, 23 gave percentages varying from 6.6 to 46.6, and one (Hybrid 75-B-F_3-109) gave 100 percent infection.

Thirty F_3 progenies were grown from uninoculated F_2 plants in the loose smut series, 8 of which proved to be entirely resistant, no infected individuals occurring in any of them; 18 progenies gave percentages of infection varying from 6.6 to 46.6, and the remaining 4 progenies (Hybrid 75-A-F_3-205, 208, Hybrid 75-B-F_3-207, and Hybrid 76-F_3-201) gave percentages of infection varying from 57.1 to 100.

Results Obtained with Ustilago levis

The data for the covered smut on the F_3 progenies are also recorded in tables 2, 3, and 4, and summarized in table 6. Thirty F_3 progenies descended from F_2 plants inoculated with loose smut were grown; of these, 8 proved to be entirely resistant to the covered smut; 17 gave percentages of infection varying from 6.6 to 42.1, and 5 (Hybrid 75-A-F_3-2, 5, Hybrid 76-F_3-9, 12, 16) gave infections from 50 to 100 percent.

TABLE 6. *Summary of Results with the F_3 Progenies of Hybrids 75-A, 75-B and 76, Inoculated with Ustilago levis*

Hybrid	F_2 Plants Inoculated with *Ustilago Avenae*			F_2 Plants Inoculated with *Ustilago levis*			F_2 Plants Not Inoculated		
	Resist.	Seg.	Sus.	Resist.	Seg.	Sus.	Resist.	Seg.	Sus.
75-A	3	9	2	7	9	1	5	10	2
75-B	—	—	—	10	7	0	6	7	6
76	5	8	3	6	5	2	3	15	1
	8	17	5	23	21	3	14	32	9

NOTE. Resistant progenies contained no infected individuals, segregating progenies had less than 50 percent of the individuals infected, and susceptible progenies gave 50 to 100 percent infection.

Forty-seven F_3 progenies were grown from F_2 plants inoculated with the covered smut. Twenty-three of these gave no infection; 21 gave infection varying from 4.1 to 41.6 percent, and the remaining 3 (Hybrid 75-A-F_3-106, Hybrid 76-F_3-113, 114) had 57.1 to 75 percent infected plants.

Fifty-five progenies were grown from uninoculated F_2 plants, and 14 of these gave no infection; 32 gave percentages of infection varying from 4.5 to 46.1, and 9 gave a percentage of infection above 50; of these 9 families, Hybrid 75-A-F_3-206, Hybrid 75-B-F_3-202, 210, 212, 213, 214, and Hybrid 76-F_3-214, gave 100 percent infection; Hybrid 75-A-F_3-212 had 63.6 percent, and Hybrid 75-B-F_3-208, 66.6 percent infected plants.

Data on the Fourth Generation

The progeny of 16 different F_3 plants, belonging to the various hybrids, were grown in the next generation (table 7). The seed of each of these progenies was divided into two sets, one of which was inoculated with loose smut and the other with the covered. The selection of the progenies was based primarily on the evidence of complete resistance in the third generation

TABLE 7. *Results with the F_4 Generation of Hybrids 75-A, 75-B, and 76*

	Inoculated with *Ustilago Avenae*			Inoculated with *Ustilago levis*		
	No. Plants	No. Inf.	Percent Inf.	No. Plants	No. Inf.	Percent Inf.
F_2 and F_3 plants inoculated with *Ustilago levis*						
Hybrid 75-A-F_4-107-1	15	0	0	15	0	0
2	15	0	0	15	0	0
Hybrid 75-B-F_4-104-1	15	0	0	15	0	0
2	13	1	7.6	15	0	0
105-1	15	0	0	14	0	0
2*	15	0	0	15	0	0
108-1*	15	0	0	15	0	0
2*	15	0	0	15	0	0
F_2 and F_3 plants inoculated with *Ustilago Avenae*						
Hybrid 76-F_3- 4-1	14	0	0	15	0	0
2	15	0	0	15	0	0
10-1	15	0	0	15	0	0
2	14	1	7.1	15	0	0
F_2 plants not inoculated; F_3 plants inoculated with *Ustilago levis*						
Hybrid 75-A-F_3-203-1	15	0	0	15	0	0
2*	15	0	0	15	1	6.6
210-1	15	0	0	15	0	0
2	15	1	6.6	15	0	0
Early Gothland	13	13	100.0	15	0	0
Monarch	14	0	0	12	11	91.6

The F_3 plants marked * had dark hulled grains; all the others had light hulls.

to both smuts. Eight progenies were grown from third generation plants which had been inoculated with covered smut; these third generation plants had also descended from F_2 plants inoculated with this same smut. Four additional fourth generation progenies were grown from F_3 plants which had been inoculated with covered smut, but the F_2 plants had not been inoculated with either smut. Further, four progenies were grown from F_3 plants which had been inoculated with the loose smut, these plants having descended from F_2 plants inoculated with this same smut.

In all of these 16 fourth generation progenies, a total of 236 plants were inoculated with the loose smut, and only 3 plants were infected. In the comparative series of experiments, 239 plants of these progenies were inoculated with the covered smut, and only 1 showed infection.

These results indicate clearly that a high degree of resistance, comparable to that of the original parents, may be observed in the fourth generation. In both the third and fourth generations, these resistant progenies have shown a very similar behavior to both of the smuts. It is thus possible to combine in definite new strains resistance to both loose and covered smut.

Inheritance of Glume Color and its Relation to Smut Resistance

As already noted, Early Gothland and Monarch differ in the color of the glumes, those of the former being classified as light, or pale yellow, while the glumes of the latter are dark, or brownish black. The data on the color of the glumes of the F_2 plants in the different series are recorded in table 8. It was not possible, of course, to obtain the glume color for infected F_2 plants on account of the fact that the floral parts were completely destroyed.

Table 8. *The Glume Color of the F_2 Plants of Crosses between Early Gothland and Monarch*

Hybrid No.	Inoculated with *Ustilago Avenae*		Inoculated with *Ustilago levis*		Not Inoculated		Total Number F_2 Plants	
	Dark	Light	Dark	Light	Dark	Light	Dark	Light
75-A	12	4	26	8	16	4	54	16
75-B	—	—	13	5	12	8	25	13
76	19	6	19	6	17	3	55	15
	31	10	58	19	45	15	134	44

Forty-one F_2 plants inoculated with the loose smut survived and produced mature grain, and 31 of these had dark glumes and 10 had light. Seventy-seven F_2 plants survived in the series inoculated with the covered smut, and of these 58 plants had dark glumes and 19 light. In the uninoculated series, 60 F_2 plants were grown, of which 45 had dark glumes and 15 light. The total number of F_2 plants observed for glume color was 178, and of these 134 had dark glumes and 44 light. These results are very close to the expectation on the basis of glume color being inherited as a single Mendelian factor. It is evident from the table that the F_2 plants belonging to the different series gave very similar results as regards glume color, and there is no evidence that plants with dark or light glumes are more susceptible to either the loose or the covered smut.

Discussion and Summary

In comparing the results obtained with the two smuts on these hybrids between Early Gothland and Monarch, the most obvious fact is the absence of similarity in the behavior of the various F_3 progenies to loose and covered

smut. Several progenies proved to be resistant to the loose smut, and some of these were entirely resistant, others showed segregation, and still others were highly susceptible to the covered smut. Hybrid 75-A-F_3-107, 203 and 210, Hybrid 75-B-F_3-104, 105 and 108, Hybrid 76-F_3-4 and 10, have shown complete resistance to both smuts. Hybrid 75-A-F_3-6, 7, 8, 10, 109, 202, and 207, Hybrid 75-B-F_3-209, and Hybrid 76-F_3-106, were resistant to loose smut, but gave with the covered smut percentages of infection varying from 6.6 to 41.6 percent. Finally, Hybrid 75-A-F_3-2, Hybrid 75-B-F_3-208, and Hybrid 76-F_3-9, resistant to loose smut, gave 64.2, 66.6, and 100 percent infection respectively with the covered smut.

In a similar fashion, a progeny segregating to the loose smut may be resistant, segregating, or susceptible to the covered smut. Various other types of possible combinations have also been found. For example, Hybrid 75-B-F_3-109 and 207 were entirely resistant to the covered smut and at the same time gave 100 percent infection with the loose smut.

It is interesting to note that no progenies entirely susceptible to both smuts appeared. This is probably due to the fact that most of the F_2 plants had been inoculated with either loose or covered smut and, if they were susceptible, no seed was formed and hence no possibility of growing the next generation. Thus, most of the progenies susceptible to one or the other smut would be prevented from producing offspring. This would tend to reduce very greatly the chance for the appearance of a progeny susceptible to both smuts. We might, however, expect such progenies to appear in the descendants of uninoculated F_2 plants. The fact that there were only 30 of these grown may have a bearing on the failure to find families entirely susceptible to both smuts.

The evidence is quite clear that the factors for resistance to loose and covered smut are independently inherited in these hybrids. Perhaps this is not surprising in view of the fact that we are dealing with two independent parasitic organisms and also with two oat varieties which show a marked difference in their behavior towards them. The results, however, are in decided contrast to those previously obtained in the study of hybrids between Hull-less and Black Mesdag (4, 5). The Hull-less variety used as one parent of these hybrids is entirely susceptible to both smuts, while Black Mesdag proved to be equally resistant. By far the larger number of F_3 progenies behaved in a similar fashion to the two smuts, so that if a progeny was resistant to one, it was resistant to the other, and if it was susceptible to one of the smuts, it was likewise susceptible to the other. There were a few progenies which gave a dissimilar reaction but, on retesting many of these, they showed a similar behavior (7).

The question may be raised as to whether there is any special significance in the variation of the percentage of infection in the families designated as segregating. Some of these gave only one infected individual out of a total of 15 or more, while others gave nearly 50 percent of smutted individuals. We would naturally expect rather wide variations when dealing with such

small numbers of plants, but it is also possible that there is some significance in the low percentage of infection in some of them.

The families classified as susceptible have also shown considerable divergence in the amount of infection. In some of them, all of the individuals were smutted, while in others one or more plants did not manifest the disease. Perhaps some of the families with infection only slightly above 50 percent should be placed in the segregating group. It will, however, be interesting to follow up still further the individuals of these so-called susceptible families which did not show any infection.

As already noted, a number of progenies (Hybrid 75-A-F_3-107, 203, and 210, Hybrid 75-B-F_3-104, 105 and 108, and Hybrid 76-F_3-4 and 10) showed complete resistance to both smuts. The F_4 generation from these families was grown, one series of plants being inoculated with loose and the other with the covered smut. Altogether, there were 16 progenies and, out of a total of 236 plants inoculated with loose smut only 3 were infected. Out of a total of 239 plants inoculated with covered smut only 1 showed any infection. These hybrids have a resistance entirely comparable to the parental varieties. The problem of the occurrence of an occasional susceptible individual in these resistant progenies is an interesting one. Since, however, we find occasional infection of Early Gothland with the covered smut and Monarch with the loose smut, it is perhaps not surprising that the resistant progenies may show an individual susceptible to one or the other.

Perhaps the most significant result is the fact that a few of the F_3 progenies are resistant to both loose and covered smut. It has been perfectly possible to combine resistance to both the smuts in new strains of oats. Further, this resistance to both smuts may be united in strains with either dark or light glumes. Probably any combination of resistance and various morphological characters could be secured.

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THE PARASITISM OF *CONOPHOLIS AMERICANA* ON *QUERCUS BOREALIS*

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Conopholis americana (L. f.) Wallr., commonly known as "squaw-root," is of peculiar interest since it is one of the few flowering plants in the northeastern United States which has become highly specialized as a root parasite.

The purpose of this study has been to determine the nature of the connection between *C. americana* and its host, and to discover the manner in which this results. A few details regarding the parasite which do not pertain specifically to the problem have been included since they were observed during the course of the investigation.

LITERATURE

The Orobanchaceae, in which *C. americana* was placed by Linnaeus (18), attain their best development in the Mediterranean region (7). Theophrastus assigned to this group the name "Orobanchaceae," literally "pea-chokers," and Micheli was the first to use the term "parasite" in the descriptions in his "De Orobanche." Cultivated crops in the southern European countries suffer considerable injury from these flowering parasites, whereas in the United States the Orobanchaceae are seldom mentioned as causing serious damage. The parasitism of *Orobanche ramosa* L. has resulted at times in extensive injury to hemp in Kentucky (11) and *O. minor* Sm. occasionally reduces the clover yields over small areas in New Jersey and Pennsylvania but the inroads of these parasites in this country have not assumed any great importance. The five additional species of this family reported as having wide distribution in the United States are of no economic significance.

The importance which the Orobanchaceae have assumed in Europe and their unique habit as root parasites have stimulated research, especially in France and Germany, and a considerable volume of European literature dealing with the family now exists. However, there is but brief mention of *Conopholis americana* since this is confined wholly to the North American continent (2, 10, 15, 27).

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After Linnaeus had described this plant and named it *Orobanche americana*, Wallroth (25) took this species as the type of his new genus, *Conopholis*, to which *C. mexicana* A. Gray has since been assigned.

Conopholis americana is mentioned in various texts on plant taxonomy, anatomy, and pathology (22, 24, 9, 13). It is included in many plant lists, in a few of which are recorded observations as to its habit of growth and evidence of parasitism. For example, Wiegand and Eames (26) offer the following information as to the occurrence and parasitism of this plant: "Dry or damp woodlands of oak and chestnut, mostly in sandy, gravelly, or stony, noncalcareous soils; apparently parasitic on the roots of oak and chestnut; scarce." Several other notes have been inserted in periodicals at various times regarding its occurrences (5, 16, 21, 23).

Chatin (4) devoted a few pages to *Conopholis* in his "Anatomie Comparée des Vegetaux Plantes Parasites" in which he describes the flowering shoot as possessing three concentric rings of vascular bundles. Miss Wilson in her paper on *Conopholis* (27) presents the results of the first detailed study of this species. She differed from Chatin in regard to the anatomy of the flowering shoot, especially the vascular portion, in which she states there are but "two concentric rows of separated, collateral fibro-vascular bundles." The only other detailed study of *C. americana* was made by Boeshore (1). He points out that the Orobanchaceae are in direct morphological continuity with the Scrophulariaceae. *C. americana* is considered to be the most reduced form, parasitically, of this series of plants. In his discussion of the morphology of the flowering shoot he agrees with Wilson that there are only two rings of vascular bundles but differs in his understanding of the tissues; he regards the bundles as bicollateral.

Methods

During this investigation, the parasitic connections of *Conopholis* were subjected to careful observation in the field and laboratory over a period of three years. Consecutive transverse and longitudinal sections were obtained both from the upright flowering shoots and the disorganized mass of tissue called the nodule, where the tissues of host and parasite commingle. These were used in the anatomical study of the parasite and in the study of the relationship which it bears to its host.

The flowering shoots and tubercles were cut into pieces approximately one inch in length and preserved in formalin-acetic-alcohol. The root of the host could be sectioned readily without imbedding; the tubercle on the other hand required imbedding in parlodion and the flowering shoots cut well in 50° paraffin. The most satisfactory stain was found to be Heidenhain's haematoxylin, followed by safranin dissolved in 50 percent alcohol. In general, methods of technique as outlined by Chamberlain (3) were used. The parlodion imbedding was done according to Lodewick's schedule (19).

The nature of the union of the parasite and the host oak, *Quercus borealis*

Michaux f., was studied in the field with the idea of locating the first symptoms of disease, and in the laboratory by germinating the seed of *Conopholis* to discover at what time and in what manner the seedling becomes attached to the oak root.

Young nodules were located by exposing the younger roots of the oak in the vicinity of the mature *Conopholis* flowering shoots. Germination of the seed was attempted by simulating the conditions under which they might germinate in nature and varying these in controlled experiments to discover the factors necessary to initiate growth. The environmental conditions were studied at three different stations in the vicinity of Syracuse, N. Y., and the results of these studies determined the course of the germination experiments. The effect upon the seeds produced by the various conditions to which they were subjected was determined by microscopic examination. The seeds were killed and fixed in 100 percent alcohol, imbedded in 50° paraffin, and sectioned to 10 microns. A light green-safranin stain was used to differentiate the structure of the seed whereas Heidenhain's haematoxylin followed by safranin brought out the cell contents to better advantage.

Distribution

C. americana is distinctly a North American species, with a wide distribution, given by Gray (12) as "southern Maine to Michigan, south to Florida and Tennessee." Although commonly classified as a comparatively rare plant (23, 26, 27), it is considered as of not uncommon occurrence in parts of Indiana (5), Kentucky (21), and western Pennsylvania (16). The plant distribution record of New York State (14) lists twenty-nine stations which are scattered rather evenly over the oak belt. This does not include a station reported on Staten Island (27) and the three stations from which the material was obtained for this study. The extent of the areas over which the plants grew thickly at the three respective stations varied from a few square rods to several acres. It would seem logical, therefore, to assume that in New York State *C. americana* is fairly frequent in those localities in which its host is abundant.

Hosts

Various investigators have stated that *C. americana* is restricted to the roots of members of the red oak group (1). For example, Jennings (16) reports it as occurring on black, red, and chestnut oaks. *Castanea* may also be a host as Wiegand and Eames (26) found "squaw-root" "apparently parasitic on the roots of oak and chestnut." During the progress of this study about forty separate plants were dug up and the roots to which they were attached traced to trees identified as *Quercus borealis* Michaux f.

Where the connections have been definitely traced, *Conopholis* has not been reported on hosts other than trees of the red oak group. At one of the stations from which material was obtained for this study the plants grew very thickly beneath a mixed stand of hardwoods and hemlock. In order to

determine the ability of the parasite to attack trees other than red oaks, a search was made for *Conopholis* nodules at points where the roots of other tree species interlaced. Twenty-two such tubercles were found which were actually in contact with the large or small roots of other trees. In every case, the roots which were parasitized were definitely identified as those of *Quercus borealis*, whereas the roots of other species in contact with the nodules showed no signs of disease. The arborescent species observed and found unparasitized were *Quercus alba* L., *Fagus grandifolia* Ehrh., *Acer saccharum* Marsh., *Acer rubrum* L., and *Tsuga canadensis* (L.) Carr.

Plant Associates

The factors determining the composition of the plant communities at the three stations under consideration varied sufficiently to result in marked differences in their respective floras.

One station was located in a narrow basin, about 50 feet wide and 900 feet long, formed on an old glacial lake beach about half way up the eastern side of Onondaga Valley and within the southern limits of the city of Syracuse, New York. The underlying ledges consisted of limestone and there were numerous pieces of calcareous rock on the surface of the soil above and below the basin. Within the basin proper acid soil conditions prevailed which were partly responsible for the plants found there. Within a radius of 25 feet of the *Conopholis* plants the following plant associates were found; "N" indicates numerous in the association.

Quercus borealis Michx. f. (N)
Tsuga canadensis (L.) Carr. (N)
Ostrya virginiana (Mill.) K. Koch (N)
Waldsteinia fragarioides (Michx.) Trattinick (N)
Monotropa uniflora L. (N)
Monotropa Hypopitys L. (N)
Quercus alba L.
Smilacina racemosa (L.) Desf.
Cerastium vulgatum L.
Carpinus caroliniana Walt.
Ranunculus acris L.
Hamamelis virginiana L.
Acer saccharum Marsh.
Viola triloba Schwein.
Prunella vulgaris L.
Veronica officinalis L.

The second station was located on a narrow strip of ground bordering upon, and about three feet above, the mean level of the Seneca River three miles west of Baldwinsville, New York. The soil was distinctly acid and moist. Within a radius of 25 feet of the two dozen plants growing there the following species were found:

Tsuga canadensis (L.) Carr. (N)
Fagus grandifolia Ehrh. (N)
Quercus borealis Michx. f. (N)
Epifagus virginiana (L.) Bart. (N)
Smilacina racemosa (L.) Desf.
Hamamelis virginiana L.
Acer pennsylvanicum L.
Acer rubrum L.
Acer saccharum Marsh.
Tilia americana L.
Fraxinus americana L.
Mitchella repens L.
Eupatorium urticaefolium Reichard

The third station was located on a high, well drained hillside on the north slope of the Mohawk River valley one mile northwest of the town of Herkimer, New York. The conditions prevailing here differed from the two other stations; first, in that the crown cover was quite open due to a logging operation which had removed approximately forty percent of the tree canopy a few years previous; second, because the soil, though kept moist by small hillside springs and a four to six inch layer of duff, had a considerably lower average moisture content. There were several hundred *Conopholis* plants here, scattered over approximately four acres. The following associates occurred within 25 feet.

Tsuga canadensis (L.) Carr. (N)
Danthonia compressa Aust. (N)
Poa compressa L. (N)
Carex digitalis Willd. (N)
Carex arctata Boott. (N)
Maianthemum canadense Desf. (N)
Quercus borealis Michx. f. (N)
Rumex Acetosella L. (N)
Podophyllum peltatum L. (N)
Waldsteinia fragarioides (Michx.) Trattinick (N)
Potentilla canadensis L. (N)
Acer rubrum L. (N)
Acer saccharum Marsh. (N)
Trientalis americana (Pers.) Pursh. (N)
Anaphalis margaritacea (L.) B. & H. (N)
Xanthium canadense Mill. (N)
Achillea Millefolium L. (N)
Hieracium florentinum All. (N)
Polystichum acrostichoides (Michx.) Schott.
Aspidium noveboracense (L.) Sw.
Aspidium spinulosum (O. F. Müller) Sw.
Lycopodium obscurum var. *dendroideum* (Michx.) D. C. Eaton
Juncus tenuis Willd.
Lilium canadense L.
Medeola virginiana L.
Corallorrhiza maculata Raf.
Fagus grandifolia Ehrh.
Quercus alba L.
Cerastium vulgatum L.
Ranunculus acris L.
Thalictrum polygamum Muhl.
Mitella diphylla L.
Hamamelis virginiana L.
Crataegus sp.
Rubus idaeus var. *aculeatissimus* (C. A. Mey.) Regel & Tiling
Fragaria vesca L.
Fragaria virginiana Duchesne
Prunus serotina Ehrh.
Rosa humilis Marsh.
Trifolium hybridum L.
Amphicarpa monoica (L.) Ell.
Oxalis corniculata L.
Tilia americana L.
Hypericum perforatum L.
Viola sororia Willd.
Viola striata Ait.
Monotropa uniflora L.
Pyrola elliptica Nutt.
Gaultheria procumbens L.
Fraxinus americana L.
Prunella vulgaris L.
Verbascum Thapsus L.
Veronica officinalis L.
Galium triflorum Michx.
Mitchella repens L.
Viburnum acerifolium L.
Lobelia inflata L.
Solidago rugosa Mill.
Aster macrophyllus L.
Aster umbellatus Mill.
Erigeron annuus (L.) Pers.
Chrysanthemum Leucanthemum L.
Prenanthes alba L.

Environmental Factors

Soil

In all three of the above stations the soil proved to be moist and was made up largely of humus; it was quite acid, as shown by tests obtained from twenty-four soil samples, all of which gave an acid reaction, averaging 5.2 pH. The samples were taken at a depth of from three to six inches, from soil immediately surrounding the nodules.

At the Herkimer station the forest floor was very uneven and there were depressions filled with humus, separated by hummocks having but a thin covering of duff. The *Conopholis* plants occurred almost without exception between the hummocks where the duff was deep. A heavy layer of decaying vegetable matter seemed necessary not only to provide the right percentage of moisture around the tubercles but also to insure the complete development of the fruit.

In order to ascertain the importance of the duff as a covering for the tubercles, three adjoining areas were plotted out in June, 1926, at the Onondaga Valley station which were treated as follows:

Plot I contained three plants; all the duff and such mineral matter as accompanied it were removed, leaving the nodules exposed to the atmosphere.

Plot II also contained three plants; the upper layer of loose duff alone was removed leaving a layer of soil, partly mineral and partly broken down vegetable matter, immediately surrounding and covering the nodules to the depth of one-half inch.

Plot III contained eight plants; as in the other instances, the duff was first largely removed but was subsequently replaced as originally found.

Observations were made at monthly intervals through the summer for three years. During the hot dry days of July and August the surface of the unprotected soil became very dry. All the tubercles of the plants in Plots I and II dried out and died during the first summer and no new tubercles were formed during the two following seasons. The plants in Plot III, on the other hand, remained alive and produced new flowering shoots each season. The plants in all the plots were attached to roots of sufficient size to insure the normal moisture supply from the host so that death, where it ensued, could not be attributed to this cause. The indications were that the scaly bark of the nodules does not afford sufficient protection against desiccation to permit of the existence of the parasite unless protected further by a moist layer of duff.

The favorable influence of the duff layer upon the development of the fruit was observed when gathering seed for germination. The fruit capsules at the upper end of the shoot were often small and contained either very few seeds or none that appeared viable. The point on the flowering shoot above which the fruit was poorly developed was quite sharply defined; this coincided in most cases with the height of the duff on the shoot. The

fully developed shoots were usually either completely covered with duff or had lifted the upper layer of this, at least a few dead leaves, as they pushed their way upward. If the shoot reached clean through the leaf mulch, the exposed portion generally failed to develop fruit. Some shoots remained completely buried in the earth and, though misshapen, developed normal capsules. One or rarely two flowering shoots of this sort often occur on the under side of a nodule. They may remain wholly hypogean and develop normal fruits or their appearance above ground is delayed; in such cases, their time of flowering is also retarded and hence shoots with flowers and others with mature capsules appear at the same time (Pl. LV, fig. 1).

Humidity

The humidity is obviously high where *Conopholis* flourishes. This condition is undoubtedly assured since the parasite is restricted to forest sites and grows best beneath a moist layer of duff.

The importance of a high humidity in the development of *Conopholis* is probably very considerable. Possibly the difference in humidity between the air in the duff layer and that immediately above it accounts for the marked discrepancy in the development of the flowering shoots. The darkening and withering of the upper portion may be due to a loss of water too rapid for the maturation of the seeds.

Light

As *C. americana* is a holoparasite, there is no apparent need for light in its development. The occasional production of flowering stalks beneath the duff is further evidence of its complete independence of light as a requirement in its metabolism.

Temperature

The wide distribution of "squaw root" both in the northern and in the southern states indicates a lack of sensitivity to the temperatures necessary for growth. The layer of duff protects the nodule from the frosts of early spring whereas the flowers are not produced until the danger from low temperatures is past; the seeds, likewise, mature in the late summer long before the advent of freezing temperatures. The time of flowering which, according to Wilson (27), is about two months earlier in the southern states, is undoubtedly influenced there by the higher temperatures of an earlier summer.

General Description of the Plant

The mature plant of *C. americana* consists of one or more fleshy flowering spikes and a portion of the tubercle from which the spikes have arisen. The tubercle is always terminal on the parasitized root although eventually it may appear lateral owing to the further elongation of a root secondary to the first, a condition comparable to the sympodial growth of stems then resulting (Pl. LV, fig. 2).

The flowering shoots vary in color from light tan to chestnut brown and

are entirely devoid of any suggestion of chlorophyll. Fifty-eight shoots from the three different stations averaged 12.8 cm. in length. The average maximum diameter of the stems was 9.5 mm. and was attained about 2 cm. above the surface of the tubercle. The nodules are very irregular, with an average maximum thickness of about 16 cm. Irregular tuberculate masses of much larger size frequently result from the fusion of two or more nodules. Occasionally very large, fantastically lobed conglomerations are formed in this way, of the type figured in Plate LV, figure 4 which consists of at least eighteen fused tubercles. At the station where this specimen was collected, many masses of the same sort were still further agglomerated and formed a platform 5 sq. m. in extent on which the tree, as it were, was seated. This tree had a D. B. H. of 90 cm. and appeared to be in a healthy condition, even though its roots supported large clumps of *Conopholis* and the majority of those near the surface were terminated by either living or dead tubercles.

In spite of the irregularities of the *Conopholis* nodule, it tends to be round, with the constantly enlarging circumference overlapping somewhat upon the host root which nourishes it. The new layers of growth on the root flare out to some extent and thus form a contact with the ever widening periphery of the nodule.

External Characteristics of the Flowering Shoot

From one-half to seven-eighths of the flowering shoot is fertile and produces flowers so closely massed that the central axis is completely hidden. The flowers are spirally arranged, short pedicellate to sub-sessile, and average approximately fifty per shoot. Each flower is in the axil of a scale-like bract of about the same length as the flower which it subtends.

Below the inflorescence the flowering shoot is clothed with triangular imbricated scales, approximately seventy in number. At the base of the shoot, just above its point of emergence from the nodule, they are greatly reduced in size and nearly equilateral. The smallest of these basal scales measure 3 mm. in length and 2 mm. in width. Higher up the shoot they become progressively larger, those immediately below the inflorescence attaining a length of 20 mm. and a width of 10 mm.

Anatomy of the Mature Flowering Shoot

The topography of a transverse section of a flowering shoot of *C. americana* is depicted in part on Plate LVI, figure 1. In order of sequence, it consists of the following tissues: 1, an epidermis of a single layer of cells; 2, a thick cortex (average width, 1.25 mm.[1]); 3, a ring of vascular tissue (average width, 1.5 mm.); 4, a broad pith (average diameter, 4.5 mm.). The observations which follow hold for sections of the mature stem taken at any point from 3 cm. to 6 cm. above its attachment to the nodule.

[1] Average of measurements obtained from fifty flowering shoots.

Epidermis

The cells of the epidermis are nearly circular in the transverse section. The walls are greatly thickened, and the outer layer of the exposed portion is cuticularized. Long, narrow-mouthed, simple pits feature all the walls, even those exposed to the outside, but in the latter case they do not extend through the cuticle (Pl. LVII, fig. 3).

Occasional stomata, apparently vestigial, are found in the epidermal layer; these lead to a small court or air chamber which is lined with cells cuticularized in a way comparable to the outer layer of the epidermis. The guard cells are functionless, the stomata being either open or closed.

Cortex

The cortical layer consists of two types of tissue. It is composed chiefly of thin-walled parenchymatous cells which appear large and round in the transverse section and are two to ten times as long vertically. The end walls of these are either perpendicular or oblique. Numerous simple pits occur in all the walls and large intercellular spaces are frequent.

Interspersed in the thin-walled cortical tissue are cells of identical shape but with much thicker walls. The simple pits here are more accentuated and are either of the normal type with parallel-margined canals or the canals may flare toward the cell lumina (Pl. LVII, fig. 5). In all other respects these sclerosed cells are comparable to those described in the preceding paragraph. Both types have protoplasts with large vacuoles.

No endodermis or pericycle could be distinguished in the mature stem.

Stele

The stele of *Conopholis americana* is of the broken siphonic type (dictyostele) with a large central pith encircled by three rings of bundles, the rings separated by intervening ground tissue. The inner ring is much less prominent than the others (Pl. LVI. fig. 1). The bundles of a given ring are separated by wide intervals of interfascicular ground tissue or may be proximate with little intervening tissue. In general the bundles of the successive rings are radially arranged.

The inner and outer rings of bundles in *C. americana* conform to the typical endarch collateral dicotyledonous type, but those of the middle ring are inverted, that is, the phloem is on the inner face, followed toward the outside by metaxylem and protoxylem in the order named. This results in an arrangement whereby the xylem tissues of the outer and middle rings face each other and the same holds true for the phloem of the middle and inner rings (Pl. LVI, fig. 2).

In considering the cell types in the bundles, it is convenient to discuss them in the order of their appearance centripetally.

The bundles of the outer ring consist of phloem, cambium, and xylem (Pl. LVII, fig. 1). The phloem is made up largely of thin-walled paren-

chyma cells. Sieve tubes and companion cells were not positively identified although a few cells appeared to have the typical shape of "sieve tube" and "companion cell" units; no sieve plates were distinguished.

The phloem parenchyma cells were small, thin-walled, angular, and generally about three times as long vertically as wide. They contain protoplasmic contents and are without intercellular spaces. Occasionally several are aligned in definite radial rows indicating secondary phloem.

There are often a few rather definite rows of rectangular cells, filled with dense protoplasm, which appear to be a greatly reduced and rather inactive cambium. In some bundles, the cambium cuts off secondary tissue consisting of as many as six rows of cells. This is a frequent but by no means a constant feature of the bundle and the new tissue is usually formed on the phloem side (distally), maturing into phloem parenchyma. A few cells are produced on the xylem side of the cambium which occasionally differentiate into pitted vessels.

The secondary xylem in a given bundle, if present, is very small in amount. It consists of short vessel-segments which may be of the pitted or reticulate type, the latter being the more frequent. The pitted vessels are equipped with orbicular or oval bordered pits in irregular, steep, uncrowded spirals, and the segments have horizontal end-walls with a solitary enlarged, bordered perforation, or the perforation may fail to form, a large bordered pit then resulting. The reticulate vessels exhibit all stages of development between the pitted and the spiral types. The lateral walls have a more or less open netted appearance and the pits in the horizontal end walls are so crowded that, as seen *en masse*, they appear reticulate.

To the inside of the restricted secondary tissue lies a well developed metaxylem which consists of scalariform-reticulate and scalariform vessels. The adjacent endarch protoxylem makes up about half of the xylem tissue of a bundle of the outer ring. In this, the spiral vessels exhibit all variations of development, from those with close spirals to those where they are far apart. Annular vessels are also present, some of which have close rings while others are partly crushed with rings which are widely separated.

The innermost cells of the bundle consist of parenchyma. There is also a considerable amount of vertical parenchyma intermingled with the vessels. All of the xylem parenchyma cells are thin-walled, angular, without intercellular spaces, and contain protoplasts with prominent nuclei.

The bundles of the middle ring of vascular tissue are similar in every way to those of the outer ring except in their centripetal order of development. Here, the exarch protoxylem occurs to the outside and is followed in order of sequence by metaxylem, occasionally a few cells of secondary xylem, a reduced cambium, and phloem consisting of small thin-walled parenchymatous cells. As in the outer vascular ring, sieve plates and companion cells could not be found.

The bundles of the inner ring which may be termed "medullary" be-

cause proximate to the pith are much smaller and about one-third the number of those in the two outer rings. The arrangement of the tissues of these medullary bundles is similar to that of the bundles of the outer ring. The outermost portion consists of a small patch of phloem parenchyma to the inside of which lie two or three xylem cells. When viewed in longitudinal section, these are seen to be reticulate, scalariform, or spiral vessels which abut directly upon the pith. No cambium was found.

The ground tissue between the bundles and the rings of bundles is made up of two types of cells, those which have remained thin-walled and those which are sclerosed. A large part of this presents the same appearance as the cortex, that is, it is made up of thin-walled ground tissue in which thick-walled cells of the same general size and shape are interspersed. The thick-walled cells, however, are more numerous and there is more or less of a tendency toward aggregation into small groups.

The most prominent feature of this ground tissue is the constant occurrence of large patches of very thick-walled fibers adjacent to the phloems of the various bundles of all three rings (solid between the medullary and middle ring) and smaller patches of thick-walled cells neighboring upon the xylem of the two outer rings (Pl. LVI, fig. 1).

The fibers of the above mentioned tracts are alike in having thick walls with simple pits of either the straight-sided or flaring-canal types, and protoplasmic contents. They differ widely from each other in form and size. Those nearest the phloem are narrow and long, the narrowest being as small as 10 microns in diameter and having a maximum length of 1200 microns. The fibers of this type have long tapering ends, a very narrow lumen, and no intercellular spaces; in addition they are often septate, having as many as six transverse walls. Farther away from the phloem, the fibers have small intercellular spaces and are progressively shorter, those occurring next to the thin-walled ground tissue merging in dimensions into the latter but retaining their thick walls (Pl. LVII, fig. 5). They are also similar in appearance to the isolated thick-walled cells of the ground tissue except that the fiber walls are even thicker and stain much more brilliantly.

The thick-walled cells in the patch adjacent to the xylem are similar to the isolated thick-walled cells in every respect except that those nearest a vascular bundle are slightly longer.

Pith

The pith is made up almost entirely of large parenchymatous cells which appear nearly isodiametric in the transverse section and are from two to five times as long as they are wide; these cells have protoplasmic contents, and large intercellular spaces occur between them. Isolated stone cells similar to those in the cortex and in the ground tissue between the vascular rings are scattered sparingly through the pith. As in the other instances cited, these sclerosed cells are similar to the cells of the thin-walled ground tissue except in wall thickness and the modification of pits entailed by it.

Anatomical Modifications of the Basal Section of the Flowering Shoot

The term "basal section of the flowering shoot" is here arbitrarily applied to that portion of the stem which lies between its place of emergence from the nodule and a point three centimeters above. Within this basal section occurs the change in anatomy from that which characterizes the main axis of the flowering shoot, described above, to that of the nodule (apparently a deformed stem structure). The morphological changes which here ensue are gradual in the upper part of this section of the flowering shoot but become abrupt near the base.

The most notable change is the increase in cambial activity which results in the formation of more secondary xylem and secondary phloem. The former consists of short vessel-segments of the scalariform-reticulate type. The secondary phloem, on the other hand, apparently is composed entirely of parenchymatous cells. With the further production of secondary tissue, the thin-walled phloem cells (primary and secondary) show signs of crushing, evidently caused by lateral pressure (Pl. LVIII, fig. 2).

The thick-walled fibers associated with the vascular strands differ only in length. They are progressively shorter until they become nearly isodiametric at the point where the stem enters the nodule. A like change results in the thin-walled cells of the ground tissue at lower levels in the basal section. The tissues in the extreme base show as complete a disorganization as is found in the nodule proper.

The Nodule

The nodule caused by *Conopholis americana* on the roots of the red oak consists almost entirely of the stem tissues of the parasite, apparently in more or less disorganized arrangement. The parenchymatous cells of the ground tissue of the flowering shoot have their counterpart here in small, thin-walled, globose cells which apparently have no orderly arrangement.

The thick-walled cells of the ground tissue of the flowering shoot shorten appreciably and progressively in the basal section and pass over into isodiametric stone cells in the nodule. The fibrous strands previously described in the upper part of the stem break up immediately after entering the nodule into large irregular masses of stone cells (Pl. LVIII, fig. 1); these aggregates of stone cells are the most prominent feature of the nodule and are responsible for the gritty feeling of the tissue between the fingers. The individual cells tend toward the globose, have very thick walls, a minute lumen with protoplasmic contents, and simple pits with long branched openings.

Through this disordered mass of parenchyma and areas of stone cells, the vascular bundles extend uninterruptedly and connect the bundles of the flowering shoot with the xylem tissues of the root of the host. The course followed by a bundle is very tortuous as it twists and weaves around the masses of stone cells. All the bundles which were traced throughout their

course were found to make definite contact with the vessels and parenchyma of the host. The irregularities of the bundles could be followed by cutting thick sections in series.

The tendency of the cambium of the vascular bundles of the flowering shoot to exhibit greater activity in the basal section increases as the bundles pass into the nodule. The area which is devoted to the primary xylem in the flowering shoot is restricted in the basal sections; in the nodule, it disappears entirely. The segments of the secondary scalariform-reticulate vessels become more numerous and shorter. The cambium produces about 12 rows of these vessels on one side and approximately twice as many thin-walled phloem parenchyma cells on the other, part of which are often crushed (Pl. LVII, fig. 2). The bundles are therefore much larger and quite different in topography than they were in the flowering shoot, in addition to their disorderly arrangement.

Connection Between Parasite and Host

The line of juncture between the tissues of the host and those of the parasite is very sharp though quite uneven. The protrusions of the parasite into the host tissues are from two to ten millimeters long. The depth of penetration is apparently of little importance except to strengthen the connection physically.

The individual vessels of the parasite penetrate only a very short distance between the tracheids of the oak, and the xylem elements of host and parasite frequently abut on each other end to end (Pl. LVII, fig. 4). The xylem parenchyma of the oak is also in direct contact both with the xylem and the phloem tissues of *Conopholis* (Pl. LVIII, fig. 1).

Host Reaction

Within a distance of 5 mm. of the nodule, the xylem tissues of the oak are changed noticeably by the irritation of the parasite. The principal modification is in the type of pitting of the tracheary tissue. The larger vessels are also replaced by long, narrow ducts and the perforations become scalariform. The xylem parenchyma is likewise more abundant.

A further reaction of the host root is evidenced by the slow infiltration of a tannin deposit into the parenchyma cells which lie adjacent to the tissues of the parasite. In a later stage of development the tracheids also show tannin deposits which, however, do not interfere at first with the life of the parasite. The older the nodule becomes, the more effectively the tracheary tissue is plugged with these tannous deposits until practically all the tissues involved are so infiltrated. When this occurs the nodule dies and slowly disintegrates, leaving a stub on the oak root the woody tissues of which are exposed but effectively protected against the attack of decay organisms.

It is not an unusual occurrence to find old nodules bearing partly developed, dead flowering shoots. An examination of the plants often discloses a

more or less complete infiltration of all host-root tissues adjacent to those of the parasite in the nodule.

Formation of Parasitic Connection

Inasmuch as *Conopholis americana* has neither roots nor chlorophyll-bearing organs at any stage in its development, it follows that the continued existence of the young seedling of the parasite depends upon its ability to form a connection with the host before the small quantity of food stored in the seed has been exhausted. Therefore, the problem of determining the procedure involved in the establishment of the parasite on its host seemed most likely of solution if young seedlings were obtained and their reactions observed in the near presence of an oak root.

Due to the small size of the seeds and also perhaps to the scarcity of seedlings young plants were not found in the soil about the old ones. To obtain seedlings, it became necessary to germinate the seeds artificially. The methods used to obtain germination were briefly as follows: 1, fresh seeds were placed at various depths in the soil near old *Conopholis* plants but out of contact with any tree roots; 2, seeds were bound with raffia to the surface of small oak roots both in the field and in the greenhouse; 3, after making incisions through the bark of several oak roots in both field and greenhouse, it was possible to place seeds in direct contact with the cambium. The openings were then drawn together with raffia; 4, a large number of seeds were placed in a leaf mulch soil around the root-tips of red oak seedlings with the expectation that any new growth of the roots would come into contact with them; 5, soil mixed half and half with cow dung was packed around the roots of young red oak seedlings which had been previously covered with moistened *Conopholis* seeds; 6, seeds from collections made during August, September, and October were placed between filter papers, some of which were moistened with tap water while others were moistened with soil water whose acidity was the same as that of the soil of the average *Conopholis* station; 7, oak root decoctions of various concentrations were poured into petri dishes, solidified, and placed in a humid atmosphere. *Conopholis* seeds were sprinkled on top of the medium and many were pressed beneath the surface; 8, scarification was accomplished with a sharp needle, the seed coats being broken without harming the embryo. The seeds were then placed between moistened filter papers; 9, mature capsules were stored in moist duff until January. The seeds were extracted and some were placed between moistened filter papers immediately while others were subjected to a freezing temperature of $-5°$ C. for ten hours and then placed between moistened filters at various temperatures; 10, a method was adapted which was used by Koch (17) for the germination of *Orobanche* seeds by root tip contact with their herbaceous hosts. The growing root tips of red oak seedlings were brought into contact with the seeds of *C. americana*. An aquarium was used as a damp chamber by fitting a glass plate over the top and constructing a

platform of galvanized wire screening 5 cm. above the slate bottom. Upon this platform, there were arranged ten Syracuse watch glasses containing wet soil brought from a *Conopholis* station. The soil was covered with white filter paper upon which two thousand *Conopholis* seeds were then placed. Above each of the watch glasses, a red oak seedling was suspended from a galvanized wire arch, by means of cords tied loosely around the stem. The seedlings and watch glasses were so adjusted that the very tips of the roots were contiguous to the seeds, making direct contact with about 25 percent of them. A few seeds and root tips were examined at the end of the first and second months and the remainder after three months.

Several of the above experiments were maintained for a period of two years with examinations made at regular intervals. A binocular microscope was used in making the external examinations and a high-power microscope for determining the orientation of tissues within the seed, after sectioning and proper staining.

The last experiment described was the only one which yielded positive results. Upon examination of the thickly matted seeds and root tips, a single seed was found which had developed a radicle. This had attained a length of about 3 mm. before it came into contact with and penetrated an actively growing red oak root tip. An attempt to get sections of the germinated seed and the point of penetration into the host root failed.

Structure of the Seed

A mature seed of *C. americana* consists of a small undifferentiated embryo and endosperm, the remains of the nucellus, and a seed coat of uneven thickness. The young sporophyte is oblong and is composed of small isodiametric cells which contain dense protoplasm, and many small varishaped granules. The remainder of the ripened oval-shaped embryo-sac is filled with the cells of the endosperm which also contain granules, similar in appearance to those present in the cells of the embryo. The seed coat is made up of very thick walled cells; there are usually two layers of these on one side of the seed and from ten to eighteen on the other. As a result the embryo is eccentrically placed, this lack of symmetry being offset by the greater thickness of the seed coat on the opposite side.

Sections cut from seeds which were in contact with the tips of living oak roots registered some departures from what has been described above. The embryo contained few or no granules and these were wanting in the cells of the endosperm immediately surrounding the embryo (Pl. LV, fig. 3). This may represent the first stage of germination. The development of the seedling was not observed further except in the one instance mentioned where the radicle actually established connection with the host root.

Discussion

One of the points of discussion raised in all former papers on *C. americana* has been the plant's specificity of host. In all cases where the parasitized

root has been traced back to the tree, the host has been found to be one of the red oaks. It has been recorded, however, that *C. americana* occurs beneath trees other than red oaks. During this study, the parasite was found only on one species of oak, namely *Quercus borealis* Michaux f., though both large and small roots of other trees were present in the soil containing numerous "squaw-root" seeds and were often in actual contact with the nodules on the oak. White oaks are abundant in central New York but they have never been reported as hosts of *C. americana*. The indications are that "squaw-root" occurs only on oaks of the Erythrobalanus group.

C. americana is found associated with plants requiring or prefering an acid soil. Tests of the pH values of the soils at the three stations from which the material for this study was obtained indicated definitely that acid conditions prevailed. In such instance the site was covered with a deep, moist leaf mulch. Furthermore, thrifty plants were unable to survive when the humus was removed from around the tubercles. The presence of a small amount of leaf mulch above the flowering shoots seemed to be favorable for the development of the seeds. This might be ascribed to the higher humidity prevailing beneath the leaves on the forest floor or to the duff-inhabiting insects often seen on the plants which possibly are agents of pollination.

The principle factors indicated as necessary for the growth of *C. americana* are the presence of a deep moist soil and trees of the red oak group. The acidity of the soil has not been shown actually to be a factor in the occurrence of "squaw-root" but merely a condition which normally accompanies a moist leaf mulch.

The anatomy of the flowering shoot of *C. americana* has been studied by three different workers with varying results. Chatin found three rings of vascular bundles present whereas Wilson and Boeshore recorded the presence of only two, but differed in the interpretation of the tissues of which they consisted. The results of this study agree with those of Chatin in that three rings of vascular bundles were observed, the bundles of the inner ring being smaller and fewer in number than those of the outer two. "Squaw-root" is similar to the closely related and associated "beech-drops," *Epiphegus virginiana* (L.) Bart., in possessing three rings of bundles. Miss Wilson has described the anatomy of the two outer rings; she states that xylem occurs on the outer side of the outer bundles and on the inner side of the inner bundles, whereas the "phloem masses of the two rows face each other." Boeshore considered the bundles as bicollateral. Neither of these workers mention a cambium.

In this study, a third explanation of the bundle structure is given. The long, narrow, thick-walled fibers (Boeshore's "hard bast") of the bundles of the outer ring and similar cells between the bundles of the middle and inner rings are considered as part of the ground tissue for the reasons that: 1, the farther they are removed from the bundle, the shorter they are in

length; at the outer edges of the tract, they merge in size and shape into thin-walled parenchyma and appear to be identical with the isolated thick-walled cells scattered throughout the ground tissue and cortex; 2, the simple pits of these cells are of the straight-sided (sectional view) and flaring-canal types, and are comparable in every way to the pits of the isolated thick-walled cells; 3, intercellular spaces which are a feature of ground tissue are present; 4, the strands of fibers, upon entering the nodule, break up into irregular masses of stone cells which bear no closer relation to the bundles than do the isolated thick-walled cells.

The vascular bundles of the outer ring consist of phloem parenchyma, cambium, and xylem. The thin-walled parenchymatous cells to the inside of the above mentioned fibers of the ground tissue are considered as phloem parenchyma because of their angular shape, the absence of intercellular spaces, and their position on the opposite side of the cambium from the xylem tissue.

The next row of cells is considered as the cambium because they appear to be flattened radially as seen in the cross-section; they contain dense cytoplasm and large nuclei, and are bounded on either side by phloem and xylem cells, aligned in radial rows.

Adjacent to the inside of each bundle of the outer ring is a tract of thick-walled cells which was described by Boeshore as "a small patch of hard bast," and by Wilson as "thick-walled parenchyma." The results of this study are essentially in agreement with Wilson on this point for the following reasons: the cells in these patches have the same dimensions and the same wall thickness as those in the other smaller patches of stone cells in the ground tissue; they have intercellular spaces; they take the same stains to exactly the same intensity of coloration, indicating a like chemical composition; finally, they break up into masses of stone cells in the nodule (that is, they do not accompany the bundle through to its connection with the host).

The tissues of the bundles of the middle ring are similar in character and in volume to those of the outer ring. The only difference lies in the reversal of orientation. The bundles of the inner ring consist of a small patch of phloem parenchyma and two or three spiral vessels toward the pith, hence they have the same orientation as the bundles of the outer ring.

In function, it would appear that the fibers in the ground tissue serve chiefly as strengthening tissues in the stem. The phloem parenchyma was apparently quite useless as a conductive tissue, the more so since it was often completely crushed in the basal section of the flowering shoot. The xylem vessels were numerous and well developed; they evidently are the main pathways for the conduction of food and water from the host.

The nodule has not received much attention from previous workers but in many ways it is the most interesting part of the plant. The present study has established the fact that the xylem tissues of the parasite continue uninterruptedly through the nodule and connect directly with the

xylem tissues of the host. The observation that the phloem parenchyma also comes in contact with the vessels and xylem parenchyma of the oak root may be of more significance than might first appear. The lack of adaptation of the phloem parenchyma of the parasite as a conductive tissue concomitant with the crushing which it normally undergoes in the basal section of the flowering shoot, would seem to indicate that the xylem functions as the main channel for the passage of both food and water from the host through the stem of the parasite. However, the parenchyma cells (in general) of *Conopholis* may have a part in the withdrawal of food and water from the host root in the region of contact from whence these materials find their way into the vessels of the parasite. It would seem logical to assume that any *Conopholis* cells could obtain dissolved foods from contiguous cells of the host because of the difference in sap concentration, as reported by Doak (8).

Another interesting feature of the nodule is the greatly increased activity of the cambium over that of the flowering shoot. The secondary growth of the irregular bundles is sufficient to account for the formation of the large, bulbous, roughened nodule and is also probably responsible for the crushing of the phloem which is quite pronounced.

The infiltration of the host cells with tannin may indicate an unusual type of disease resistance, at least for a parasitic flowering plant. The resistance of a host to flowering plant parasitism, however, is a subject which has received comparatively little attention.

Inasmuch as only one seed was observed to germinate and to establish connection with the host, it is unwise to accept this method of attack as indicative of the conditions which usually prevail. Comparison with the seedling development of other members of the Orobanchaceae indicates, however, that the seedling in this instance conformed to the usual method of attack followed by these parasites. For example, Koch (17) states that contact must be made between the host root and the seeds of *Orobanche ramosa* L., *O. minor* Sm., and *O. Hederae* Duby before germination can take place. Garmen (11) finds that the seeds of *Orbanche ramosa* L. must be in the presence of the fibrous roots of the host plant before they can germinate; he states further that "No other roots have any effect upon the dormant seed." Meehan (20) infers that these conditions are important for the germination of *Orobanche uniflora* L. seeds. Cooke and Schively "surmised that for successful germination, the seeds of *Epiphegus virginiana* (L.) Bart. require contiguity with the beech root"; they were not, however, able to get the seeds to germinate.

In this study the results of a variety of germination tests involving over 22,000 seeds were negative except where the seeds were in proximity to the host roots. In this one instance, the embryos of several showed a modification in cell content and absorption of food from the endosperm, and one seed developed a radicle which penetrated the root tip of a red oak seedling.

In view of the similar conditions observed as necessary for the germination of the seeds of other members of the Orobanchaceae, it seems highly probable that the seeds of *C. americana* germinate only in the presence of the host root. The tests carried out in this study also would seem to indicate that germination ensues only when the seeds are in close proximity to root tips in active growth.

Summary

1. *Conopholis americana* was found in central New York on but one host, *Quercus borealis* Michaux f. The associated arborescent species, *Quercus alba* L., *Fagus grandifolia* Ehrh., *Acer saccharum* Marsh., *Acer rubrum* L., and *Tsuga canadensis* (L.) Carr. were unparasitized, even where their roots were contiguous to the diseased parts of red oak.

2. The distribution of *C. americana* is restricted principally by two limiting factors, namely, the occurrence of a suitable host (a member of the red oak group) and a deep moist soil.

3. The axis of the flowering shoot has three rings of collateral bundles; the outer and the inner rings have phloem to the outside and xylem to the inside whereas the tissues of the middle ring are inverted.

4. The xylem of the outer ring of bundles is endarch in its development, that of the middle ring exarch, while in the inner ring the differentiation of the tissues is insufficient to indicate with certainty the direction of xylem development.

5. The bundles consist of xylem parenchyma, vessels, cambium, and phloem parenchyma; through parasitism the sieve tubes have apparently been eliminated.

6. The thick-walled fibers in the ground tissue adjacent to the phloem have not been considered as part of the bundle, and a similar interpretation has been made in the case of the thick-walled cells proximate to the xylem.

7. In the base of the flowering shoot, cambial activity proceeds longer than at points higher up the stem, resulting in a larger volume of secondary xylem and phloem. In the nodule proper, there is little primary xylem but this is offset by a pronounced increase in the amount of secondary tissue resulting from a very active cambium which is sufficient to explain the excessive hypertrophy; the nodule consists largely of the stem tissue of the parasite.

8. The vascular bundles can be traced from the flowering shoot through the nodule directly to their point of juncture with the xylem of the oak root.

9. The infiltration of appreciable amounts of tannin in the tissues of the host adjacent to the area of attack are indicative of the resistance which the host offers to the inroads of the parasite.

10. The present study favors the assumption that the seeds of *C. americana* germinate only in proximity to the root tips of the host, and only when active growth is in progress.

It is a pleasure to express my appreciation to Dr. H. P. Brown for his suggestions, encouragement, and criticism during the progress of this investigation.

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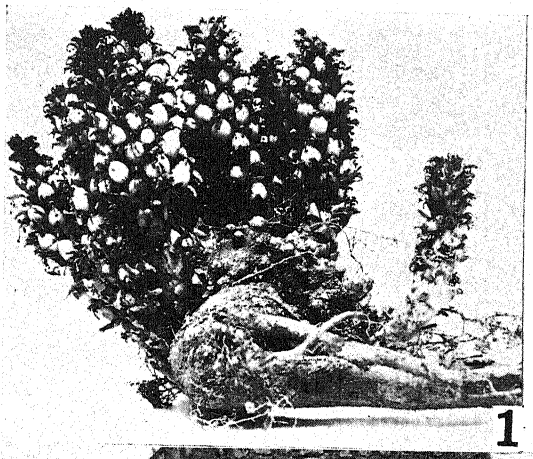
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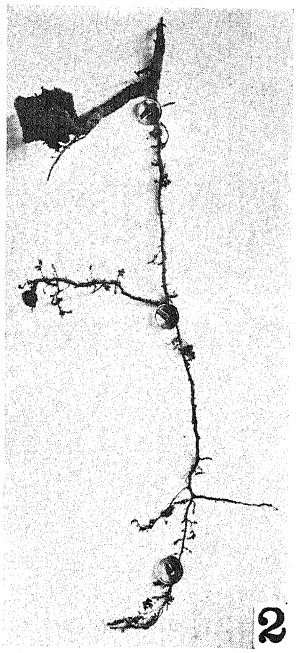
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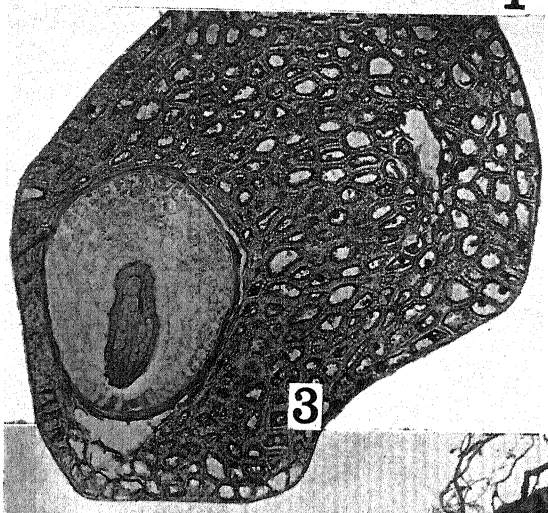
DESCRIPTION OF PLATES

PLATE LV

FIG. 1. A nodule bearing flowering shoots of *Conopholis americana* on a root of *Quercus borealis* Michaux f. All of the shoots are in fruit with the exception of one in flower which arises from the under side of the nodule. × ¼.



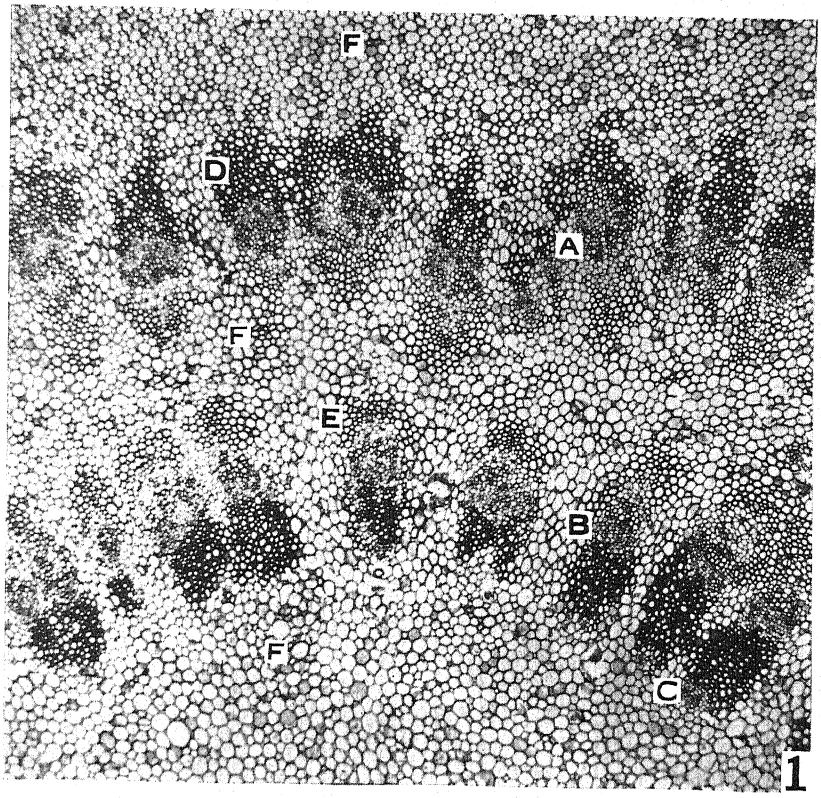


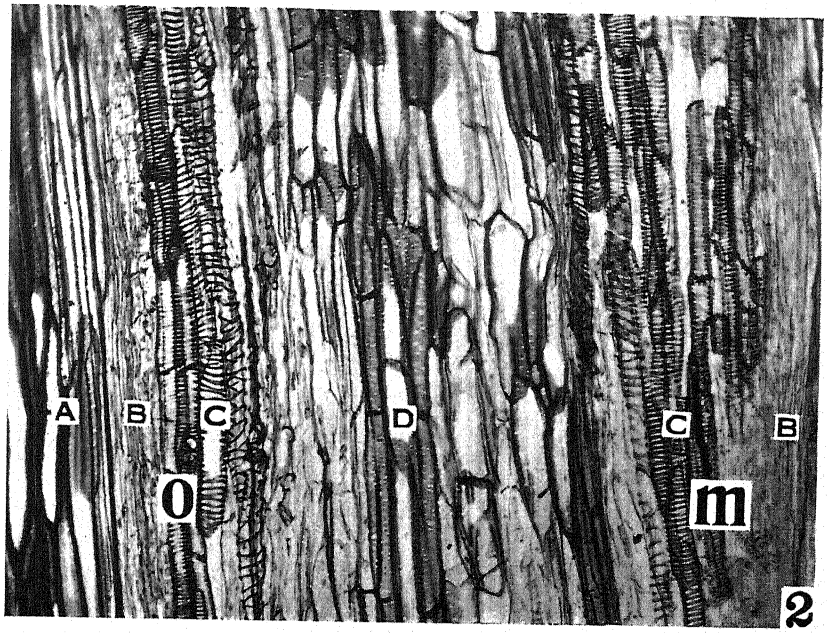




PERCIVAL: CONOPHOLIS

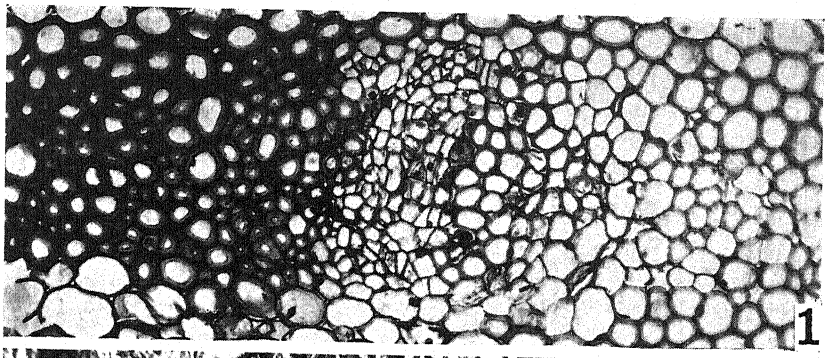


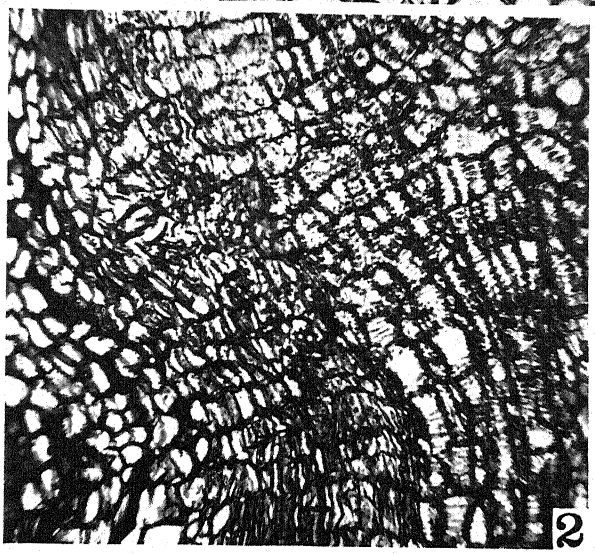


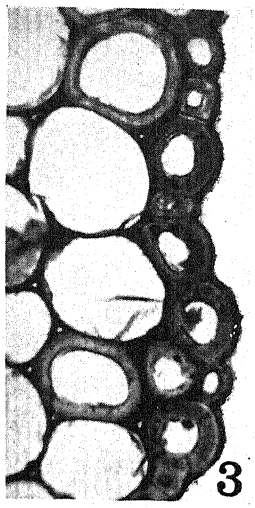


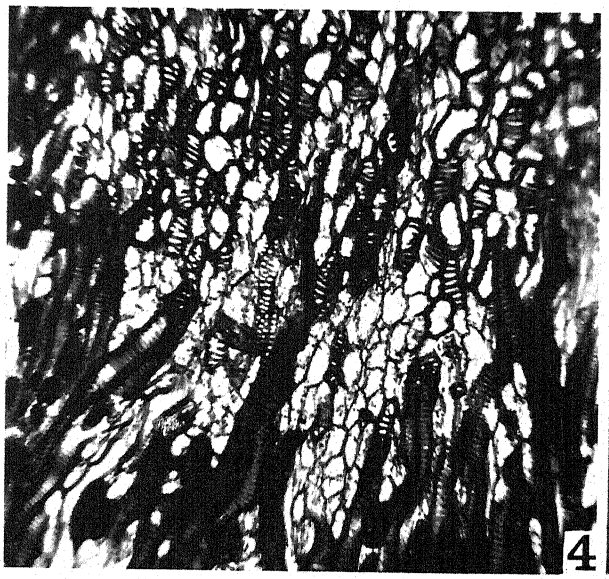
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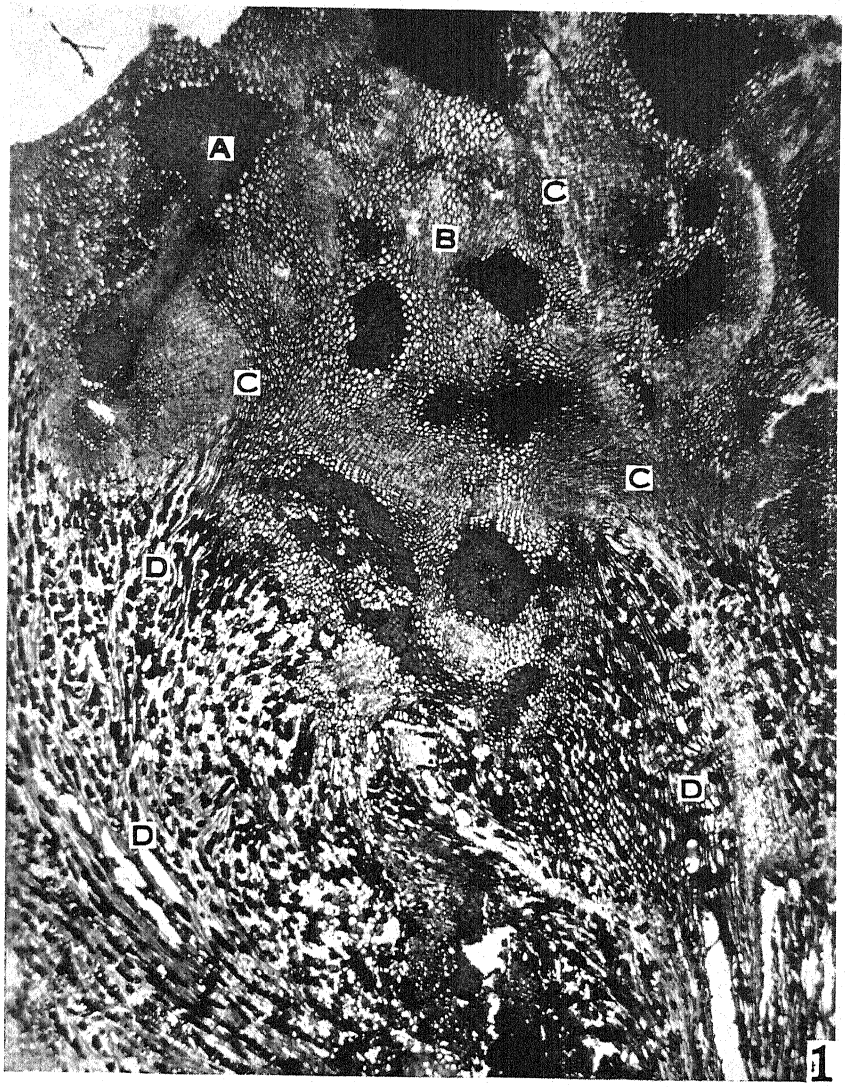

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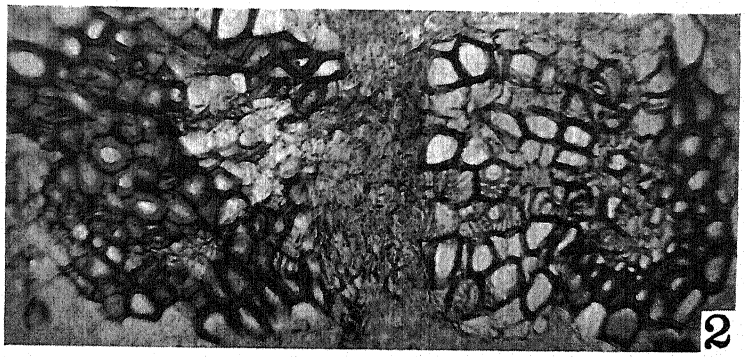

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PERCIVAL: CONOPHOLIS

FIG. 2. Young *C. americana* nodules on a red oak root showing the development of secondary roots. × ¼.

FIG. 3. Longitudinal section of a *C. americana* seed which had been in contact with a growing oak root tip. × 60.

FIG. 4. Mass of eighteen fused tubercles only two of which were bearing flowering shoots. × ⅕.

PLATE LVI

FIG. 1. Part of a transverse section of the *C. americana* flowering shoot, taken 3 cm. above the surface of the nodule. This includes a section of the vascular region containing three rings of bundles together with neighboring portions of the cortex and pith. *A*, outer ring. *B*, middle ring. *C*, inner ring. *D*, fibers of the ground tissue adjacent to the phloem. *E*, thick-walled cells of the ground tissue adjacent to the xylem. *F*, thick-walled cells either singly or in groups bearing no relationship with the vascular bundles. × 35.

FIG. 2. Longitudinal section of vascular bundles of the outer and middle rings in a flowering shoot of *C. americana*, 2 cm. above the surface of the nodule. *O*, bundle of the outer ring showing endarch development of the xylem. *m*, bundle of the middle ring showing exarch development of the xylem. *A*, fibers of the ground tissue adjacent to the phloem. *B*, phloem. *C*, xylem. *D*, thick-walled cells of the ground tissue adjacent to the xylem. × 120.

PLATE LVII

FIG. 1. Transverse section of a typical vascular bundle of either the outer or the middle ring of a flowering shoot of *C. americana*, 3 cm. above the surface of the nodule. A weak development of cambium is evident. From left to right, the tissues are as follows: thick-walled fibers of the ground tissue adjacent to the phloem, phloem, cambium, xylem, and thick-walled cells of the ground tissue adjacent to the xylem.

FIG. 2. Longitudinal section of a vascular bundle in a nodule of *C. americana* showing secondary growth. On left, phloem parenchyma, partially crushed. On right, xylem, composed of short vessel segments. × 180.

FIG. 3. Transverse section of the epidermis and adjacent cortical cells of *C. americana* showing the simple pits in the outer walls. × 350.

FIG. 4. Longitudinal section of xylem tissues of a nodule of *C. americana* and a root of *Quercus borealis* Michaux f. showing the juncture of parasite and host. Above, vessels and parenchyma of *C. americana*. Below, vessels and parenchyma of host. × 180.

FIG. 5. Longitudinal section of a thick-walled cell of the ground tissue showing the flaring-canal type of simple pit. × 300.

PLATE LVIII

FIG. 1. Longitudinal section of a portion of a *Conopholis* nodule and the host root. *A*, stone cells of parasite. *B*, parenchyma cells of parasite. *C*, vascular bundles of parasite. *D*, xylem parenchyma and vessels of the host. Many of the parenchyma cells of the host are filled with tannin. × 35.

FIG. 2. Transverse section of a vascular bundle in the basal section of the flowering shoot of *C. americana*. From left to right, the tissues are as follows: fibers of the ground tissue adjacent to the phloem, phloem (crushed), xylem, thick-walled cells of the ground tissue adjacent to the xylem. × 170.

THE FORMATION OF THE ACHROMATIC FIGURE IN *SPIROGYRA SETIFORMIS*

F. McALLISTER

(Received for publication June 11, 1931)

The achromatic figure of *Spirogyra* is generally believed to develop from the cytoplasm. Strasburger (28, 29, 30) came to this conclusion after exhaustive studies on several of the larger Spirogyras. He believed that the polar caps, from which the spindle arises, develop as transformations of the spongy cytoplasm which is abundant at the bases of the suspending strands. The fibers which are formed in the polar caps were believed to penetrate the nuclear membrane and enter the nucleus in the later prophases. Somewhat previous to the metaphase stage the nuclear membrane was believed to disappear. Tangl (31), Mitzkewitsch (17), Van Wisselingh (35, 36), and Berghs (2) are in essential agreement with Strasburger with respect to the origin of the achromatic figure. Van Wisselingh differed in that he believed that the fibers, extending out from the polar cap, do not penetrate the nuclear cavity but extend around the periphery of the nucleus. Flemming (9) believed that, although fibrillar polar caps are formed in early prophase, they later disappear and the spindle is formed entirely within the nuclear cavity, the nuclear membrane persisting into the anaphases. Meunier (15) was of the opinion that the achromatic figure arises partly from the cytoplasm and partly from the nucleus.

Most of the work referred to above was with large-celled species, the identity of which is often uncertain. The earlier work was with crude fixatives, such as alcohol or dilute chromic acid, and the cells were examined entire. In view of the lack of uniformity of materials and technique it is surprising that such close agreement as to the origin of the achromatic figure has been reached.

The material which has been used as a basis for this study is closer to *Spirogyra setiformis* than to any of the other large celled forms, although not conforming any too closely to Kützing's (11) description of that species as given by Petit (23). The cells average about 85 microns in diameter and are often slightly more than twice that length. The plastids usually number five although four seems not uncommon. The spiral chloroplasts are closely crowded and often extend at least twice around the cell. Material collected in Wisconsin conforms much more closely to the measurements of *S. setiformis* as given by Petit than material collected about Austin, Texas. While the Wisconsin material has a diameter usually greater than 100 microns and that from Texas does not reach 90 microns in

diameter, the proportion of the length of the cell to its width and the number and length of the plastids and their crowded condition is very similar in both lots. Other material from Colorado is very similar in size and in structural details to the form common in the Austin region. Apparently the species is quite variable or else at least two quite distinct forms are included in the species. Since zygospores are not to be found in material in which the cells are multiplying rapidly, indentification must necessarily be based upon details of vegetative structure. Zygospores very similar to those described for *S. setiformis* have been found in Austin material which has a vegetative structure very close to that of the material used for this study.

Merkel's solution has been found the most satisfactory fixing agent. After fixation the filaments of the algae are arranged as nearly parallel as possible and folded in lens paper. This prevents tangling during washing and through the subsequent processes up to infiltration. The material is carried through all the steps, from dehydration to infiltration, in a short diffusion shell so that the changes are gradual and, as a result, distortion is reduced to a minimum. After washing, the material is placed in the diffusion shell, in water, and carried through the dehydrating and clearing agents as follows; 95 percent alcohol, 12 hours; absolute alcohol, 6 hours; chloroform, 6–8 hours; solution of one part paraffin in two parts chloroform, about two days. The material is then transferred to a small evaporating dish and the chloroform evaporated slowly. This is usually accomplished by keeping at about 33° C. for one day and then transferring to the paraffin oven at about 56° C. for another day. Of course the length of time required depends upon the amount of the paraffin solution at the beginning. During infiltration the paraffin solution must be kept stoppered at about 33° C. to prevent its becoming solid. The lens-paper wrapping is removed when the paraffin solution is put into the evaporating dish.

Considerable living material has been examined for mitotic figures from time to time. The crowded spiral chloroplasts interfere greatly with observations of nuclear phenomena. The prophases are easiest to find, either due to the large size of the nucleus at this stage or possibly due to the longer duration of this phase.

Enlargement of the Nucleus

The first evidence of approaching mitosis in *Spirogyra setiformis* is seen in the rapid change in the size and shape of the nucleus from the form of a flattened sphere, which is characteristic of the growth period of the cell, to an ovoid body having a longitudinal dimension one-fourth greater than its transverse diameter. During this period the transverse diameter remains unchanged. Conspicuous accumulations of cytoplasm are also formed at the poles of the nucleus at this time. Examination of living material during this period of growth shows very conspicuous movement,

both in the nucleus and in the cytoplasmic strands which suspend it. Since the movements do not seem to have any definite direction they are probably to be interpreted as connected with rapid absorption of water or some change in the colloidal state of the protoplasm.

Previous to this enlargement, the suspending strands of cytoplasm are noticeably broadened at their bases, but no definite polar aggregations are recognizable. However, by the time the nuclei are noticeably longer than broad, conspicuous accumulations of protoplasm are to be seen at the poles. These accumulations appear as enlargements of the bases of the suspending strands coalesced to form irregular caps of spongy protoplasm.

Very soon that part of the polar aggregation of cytoplasm which is in contact with the nucleus becomes less spongy and shows a striated condition which begins at the nucleus and extends outward (Pl. LIX, fig. 1). These striated regions lengthen to form crescent-shaped areas. They are at first not distinctly separated from the remaining spongy cytoplasm (fig. 2) but, as the striations grow longer, a distinct line of separation is to be observed (fig. 3). At their maximum size the striated polar caps are about one-fourth the longest diameter of the nucleus.

With the enlargement of the striated cap area there is a decrease in the volume of the spongy cytoplasm, although this does not become very noticeable until the period of the maximum development of the striated zone (fig. 4). This is due to the fact that the striations begin to appear while the polar accumulations are still incomplete.

This extra-nuclear growth and differentiation has taken place during the period of rapid growth of the nucleus. Apparently neither the growth of the nucleus nor that of the cytoplasm has been at the expense of the other. If material from the nucleus has taken part in the development of the striated polar caps its loss has been more than compensated by the rapid growth of the nucleus.

The entire cells of *S. setiformis* are not easily studied because of the closeness of the spiral chloroplasts. While no striations are to be observed in the polar caps in living material, neither could the boundary line separating the striated portion of the caps from the spongy protoplasm be clearly seen.

From the early prophase the nuclear membrane is seen to be more or less distorted in the regions where it is in contact with the striated polar caps (Pl. LIX, figs. 1, 2, 6). The flanks of the nucleus do not show this distortion. This wrinkled, distorted appearance of the nuclear membrane in this region is to be seen until late prophase. Berghs (2) interprets it as due to the pushing action of the fibers of the caps.

When the striated zone at the poles of the nucleus is fairly distinct, the outline of the nucleolus has become uneven and large granular portions become separated from it and are scattered through the nuclear cavity (fig. 3). The nucleolus seems at first to enlarge. This appearance is due

probably to its loose, irregular outline, or it may be due to actual loosening and swelling of the nucleolus by vacuolization. Whatever the cause, irregular scattered granular material becomes conspicuous in the nuclear cavity and the irregular mass of nucleolar material decreases noticeably in size. The author has not observed any cases in which the nucleolus has entirely disintegrated. The residual mass together with the scattered granular material moves to the equator with the reticular material and forms the diffuse equatorial plate (fig. 6). Berghs (2) is of the opinion that the nucleolus persists throughout mitosis in *Spirogyra*. From his figures no part of it, except the chromosomes, escapes into the nuclear cavity. "Il ne disparait à aucun moment de la cinèse."

Development of Spindle from Polar Caps

By the time the striated polar caps have reached their maximum size scattered striations can be seen in the interior of the nuclear cavity (fig. 4). They are first seen adjacent to the polar cap and later extend toward the equatorial region of the nucleus (fig. 6). Some of the striations seem to be continuous with others outside of the nucleus and seem thus to have penetrated the nuclear membrane. Other striations do not have this continuity through the nuclear membrane. These phenomena have been observed by Strasburger, Berghs, and others, who have interpreted them as showing penetration of the nuclear membrane by the developing spindle fibers.

That the striations are not simple fibers is seen from transverse sections of late prophase and early metaphase stages. A cross section of the polar cap at a stage in which the outline of the nucleolus has become very irregular and ragged, similar to that shown in figure 3, shows a mesh-like appearance (fig. 5). Circular unstained areas are surrounded by thin boundaries of protoplasm, and thickenings suggesting those characteristic of collenchyma tissue are seen at the angular regions caused by the junction of three or more of the circular areas. That the appearance is not due to coarse spongy protoplasm is of course seen in longitudinal sections of these areas. The striations are therefore not distinct strands but are the elongated protoplasmic masses which occupy the angular spaces between the parallel cylindrical unstained regions of the achromatic figure. When observed in longitudinal sections these elongated thickened regions have the appearance of fibers.

Evidence that the entry of the striated condition into the nuclear cavity is not that of isolated fibers but of a fairly compact front of a protoplasmic change is seen in the fact that the scattered nuclear material, a conspicuous part of which is from the disintegrated nucleolus, moves ahead of the advancing striations toward the equatorial region (fig. 4). All of the granular, stainable material of the nucleus moves toward the equator and forms the rather diffuse "equatorial plate" (fig. 6).

Transverse sections of the metaphase spindle show conditions similar to

those to be seen in cross sections of the polar cap areas. A collection of circular unstained areas is seen, each surrounded by a thin layer of slightly stained protoplasm (fig. 10). At the points of junction of several of the circular areas protoplasmic thickenings are observable. The honeycombed mass is surrounded by a thin layer of homogeneous protoplasm. Between the nuclear membrane and this striated area is usually a clear zone which does not stain to any noticeable extent.

As the striated condition becomes more evident in the nuclear cavity there is a decrease in the size of the polar caps (fig. 6). The striated condition finally disappears from them and the remaining protoplasm of the crescent-shaped striated caps becomes a part of the spongy protoplasm at the poles of the achromatic figure (fig. 7).

The decrease in the size of the polar caps may suggest that some of its materials have passed into the nuclear cavity. There has been very little change, however, in the apparent volume of the nucleus during this change in the caps. The volume of the nuclear cavity at metaphase is very little greater than that of the prophase nuclei. It seems much more reasonable to conclude that the caps have returned to the more compact colloidal state prevailing before prophase.

At the time when the stainable material forming the diffuse equatorial plate is at its most contracted stage, the nuclear membrane is still intact (fig. 7), with most of the striations of the spindle extending clear to the poles of the nuclear cavity. The fibrous appearance has disappeared from the protoplasmic caps and the total volume of the cytoplasm at the poles has decreased. The nuclear cavity has elongated so that it is nearly twice as long as before the entry of the fibrous appearance into the nuclear cavity. This elongation has been accompanied by a distinct narrowing of the nuclear cavity at the polar regions, while no marked change in the diameter of the figure occurs at the equator at this period (fig. 8). This narrowing of the cavity at the poles causes the nuclear figure to appear distinctly bulged in the equatorial region.

The granular mass of the equatorial plate is now seen to be widening in a longitudinal direction, and very soon it separates into the two masses characteristic of the early anaphase stages (fig. 9). Before the separation of the chromatic masses has become pronounced, the striated condition of the spindle no longer extends to the nuclear membrane at the poles (figs. 8, 9). Instead, a distinct clear area, free from stainable material, is to be seen at the ends of the nuclear cavity. The "fibers" of the spindle lie almost parallel and do not at any time converge in the polar regions to form anything comparable to a unipolar spindle.

At the stage marked by the separation of the mass of equatorial material into two halves, that is, the beginning of the anaphases, the bulging of the achromatic figure in the equatorial region becomes more pronounced and the rupture of the surrounding membrane in this region soon follows (fig. 9).

In longitudinal sections, apparent striations can be seen extending out from this ruptured region into the surrounding vacuolar area of the cell. The striations are not continuous on both sides of the equator, indicating clearly that a ruptured condition of the nuclear figure persists here and that no definite membrane has been reformed. The bulging and bursting of the membrane surrounding the spindle in the equatorial region is probably an expression of the local weakening of the osmotic membrane at this region. The striations of the nuclear figure do not extend clear to the membrane in the polar regions but end in a clear vacuole-like region (figs. 8, 9).

This stage has been observed a few times in living material. While no membrane is to be seen in the equatorial region at this time, there is no conspicuous evidence of the dispersal of cytoplasmic material in the region where bursting is indicated in fixed material. It is possible that the appearance of a broken membrane here is due to the effects of fixation. It is nevertheless clear that the nuclear membrane disappears first in the equatorial region at the beginning of the anaphases. The vacuole which arises between the chromatic masses in the later anaphases and early telophase is not surrounded by any remnant of the nuclear membrane but by a newly formed membrane.

In living material of *Spirogyra Weberi*, Behrens (1) observed a vacuole with homogeneous contents in the space between the separating chromatic masses in late anaphase. He believed that this vacuole arose from smaller vacuoles which formed in the central spindle area in early anaphase. The enlargement of the vacuole was believed to push the chromatic masses apart.

From fixed material of an unidentified *Spirogyra*, Tangl (31) describes and figures a swollen unbroken sac between the anaphase chromatic masses. He calls this sac the "Verbindungsschlauch." Strasburger (30) also calls attention to the vacuolate condition of this central strand.

It is clear that in *S. setiformis* the vacuole between the developing daughter nuclei arises *de novo*, and is not a residue of the nuclear sap surrounded in part by nuclear membrane. Behrens's interpretation of its formation conforms very closely to the conditions observed in *S. setiformis*.

The equatorial plate is clearly made up of at least two kinds of material. A finely granular material, staining grayish in Heidenhain's iron-alum-haematoxylin and a light violet in Flemming's triple stain makes up the ground substance. Minute bodies form a distinct plate-like layer in the center of the more diffuse equatorial ground substance (figs. 6, 7). These latter bodies stain deep black or deep violet with the above mentioned stains. It is very probable that they are chromatic material and should be called the chromosomes. Similar bodies have been so called by all earlier investigators of mitosis in *Spirogyra*. The lighter staining ground material seems to be non-chromatic and is probably of nucleolar origin.

The combined stainable material of the equatorial plate, even before the period of the greatest condensation, shows a definite appearance of

being arranged in diffuse, elongated, cylindrical masses, extending parallel to the long axis of the nuclear figure (figs. 7, 8). In the anaphases, this appearance of elongated masses becomes very definite so as to give the impression of large, diffuse chromosomes passing to the poles of the spindle (fig. 9). They are not chromosomes, of course, in the usual conception of the term, but are made up of a large amount of nucleolar material in which is imbedded a very small portion of chromatic material, forming together diffuse elongated cylinders. It is doubtful if they become distinct from one another until the late telophase stages. It is quite possible that these masses would be termed chromosomes if their outlines were more definite.

It is obvious that one and the same force must be responsible for the movement of these combined cylindrical masses. If contractile fibers are present they would be unable to cause the movement of these loosely aggregated masses. The shape of the masses is not such as one would expect of materials moving under the influence of contractile fibers. It is difficult to understand how such loosely connected material could move together except under the influence of some general motive force, as possible protoplasmic flow.

Cytokinesis

As the chromatic masses separate in the anaphases, a somewhat scant skein or weft of apparent protoplasmic strands is to be observed between them, in the position normally occupied by the phragmoplast in mitosis in higher plants (Pl. LX, fig. 11). This is the "Verbindungsschlauch" of Tangl to which reference has been made. There may be some question as to whether this material is at first strictly comparable to the phragmoplast. Since however it develops ultimately into a cell-plate-forming structure it seems proper to give it the name "phragmoplast" from its inception. The strands are coarse and irregular and do not have the uniformity and delicacy of the striations of typical phragmoplasts. They appear, at first, as residual protoplasm left over from the layer which enveloped the nucleus.

Cross sections of the central part of the anaphase figure at a stage similar to that shown in figure 11, in which the chromatic masses have moved about a half the distance to the poles or about 8 or 10 microns from the equator (fig. 12), indicate that the apparent fibers between the separating chromatic masses are due to vacuolate protoplasm surrounding a central mass of what appears to be elongated vacuoles. The appearance of fibers is due to elongated aggregations of cytoplasm, mainly at points of junction laterally of several of the elongated vacuoles. That the vacuoles must be greatly elongated is seen in longitudinal sections in which no transverse divisions are visible.

The vacuolate central region of the phragmoplast seems to be an area of cell sap shut off from the main vacuole of the cell, forming thus a distinct osmotic system in the interior of the cell. In the later anaphases irregular vacuolate swellings may be observed in the central region in longitudinal

sections (fig. 13). These are probably due to fusion and enlargement of the vacuolate interior of the earlier phragmoplast. As the telophases are initiated a noticeable single vacuole is usually to be observed in the phragmoplast (fig. 14). This single large vacuole is usually asymmetrical, pushing out more to one side than the other, very probably due to differences in the thickness of the surrounding protoplasm.

Further stages indicate that the above described vacuole continues to enlarge, pushing the protoplasmic membrane out and causing the enclosed vacuole to assume an ovoid form, joining as it expands with some of the cytoplasmic strands which support the nucleus. The central region of this thin distended layer of cytoplasm finally comes into contact with the layer of protoplasm at the lateral wall. Strasburger saw these vacuoles as early as late anaphase and followed their development nearly to the time of their junction with the lateral wall. It is at this point of contact with the lateral wall, during early telophase, that the first evidence of cell plate formation has been seen by the author. Strasburger (29, 30), Tangl (31), and Moll (19) have described cell plate formation as beginning as early as the middle anaphases. In *S. setiformis* it has not been observed earlier than early telophase.

The relation of the protoplasmic layer to the developing diaphragm is difficult to follow in the earlier stages because of the presence of the crowded chloroplasts in the region. As the diaphragm develops, the chloroplasts are pushed away from the lateral wall toward the center of the cell, and often do not break in front of the advancing septum until it is nearly one-fifth formed (fig. 15). This obscures the development of the diaphragm since it is almost entirely covered by the stretched chloroplasts. After the chloroplasts have separated into two parts, the diaphragm grows away from them and its development can be followed with ease. The swollen sac-like phragmoplast is then seen to be almost spherical, with definite accumulations of striated cytoplasm in the equatorial region, immediately in advance of the developing septum (fig. 15). Median cross sections of cells of this stage, the age of which is quite accurately determined by the condition of the daughter nuclei, show a distinct layer of protoplasm surrounding a clear central area (fig. 16). This gives convincing evidence that the sac-like phragmoplast is surrounded by a continuous membrane. The shape of the sac is such as can be satisfactorily explained only as being due to turgor. During later stages of cytokinesis it becomes increasingly clear that the cell sap enclosed within the hollow vesicle of the phragmoplast is osmotically distinct from the surrounding main vacuole of the cell. Very often the sac bulges on each side of the advancing diaphragm, indicating a state of turgor (fig. 17). In a part of the division figures the daughter nuclei have noticeable depressions in the parts in contact with the vesicle of the hollow phragmoplast (figs. 17, 18). Moll (19) shows such a depression in his figure 40 although he makes no reference to it.

As has been mentioned, the cytoplasmic layer is very much thickened in the region of the developing diaphragm (figs. 15, 17). In living material this thickening cannot be distinguished with certainty so that the enclosed vesicle is somewhat indistinct. In fixed material these protoplasmic thickenings near the diaphragm show fairly regular striations quite similar to those seen in earlier stages of the formation of the achromatic figure except that they are finer and more delicate (fig. 17). These striations show especially clearly in tangential sections of the vesicle at the region of membrane formation (fig. 19). A line of separate thickenings is often to be seen just in advance of the developing membrane (fig. 19). These usually appear as elongated thickened parts of the striations in this region. They give a very definite impression of the accumulation of the finely granular material of the strands, possibly the gel phase, in that part of the phragmoplast in front of the region of membrane formation. The thickenings are apparently but a stage in the condensation of cytoplasm in the region preliminary to cell plate formation.

The cell plate appears as a compact layer of granular material and does not show any signs of splitting until the septum has been completed for some time.

The formation of the cell plate between the two daughter nuclei has much in common with the process as seen in the higher plants. The hollow phragmoplast shows the striated condition characteristic of the latter plants, with thickenings of the striations ahead of the region of cell plate formation. There seems little essential difference between the process in *Spirogyra* and that in the higher plants other than the region in which plate formation is initiated.

Discussion

Robyns (24) has described the formation of the polar caps in *Hyacinthus* and *Vicia* as due to the passage of nuclear sap outward at the poles of the nucleus, where it forms crescent shaped caps. The nucleus decreases in longitudinal diameter and becomes noticeably flattened in a plane parallel with the shorter axis of the cell. The chromatic material, by this flattening, is brought nearer to the equator. The nuclear membrane persists for a time and a limiting membrane seems to be present at the point of contact between the karyolymph sap and the cytoplasm. According to this interpretation the achromatic figure is entirely of nuclear origin.

From the evidence at hand it is seen that in *S. setiformis* the polar caps are formed at a period of rapid growth of the nucleus. The passage of karyolymph into the caps therefore could occur only if the increase of volume is such as to account not only for the increase in the volume of the nucleus but also for the growth of the caps. It seems very improbable that, in the earlier phases at least, the nucleus could supply any material to the developing polar caps. The distortion of the nuclear membrane at the region of contact with the polar caps nevertheless suggests a possible

interchange between nucleus and polar caps. While the distortion is very probably due to the action of the fixative it indicates that the membrane here is more sensitive to the action of the reagents. Whether such sensitiveness can be interpreted as due to increased permeability is rather doubtful.

Berghs (2) has figured a similar distorted condition of the nuclear membrane at the poles of the nucleus at the time of the entry of the striated condition into the nuclear cavity. He ascribes the unevenness to the action of the fibers in penetrating the nuclear membrane. The fibers are regarded as entering the nuclear cavity by pushing against the membrane and depressing it locally and finally penetrating it. Fibers are shown at the bottom of the depressions. This interpretation is not applicable to *S. setiformis* since the irregularities in the membrane at this region are present long before the appearance of fibers in the nuclear cavity and are to be seen while the polar caps are in early stages of their development. That fibers are actually present is very doubtful and that they are present with sufficient rigidity to penetrate the nuclear membrane seems quite out of the question. The hypothesis of Meves (16) and others, that the fibers of the achromatic spindle are pushing organs, has received very little notice. The formation of the fibrous condition within the nuclear cavity in *Spirogyra* is without doubt due to essentially the same causes as have brought about this condition in the polar caps. Both undoubtedly involve colloidal changes in the protoplasmic medium and while, due to the influence of Strasburger and his kinoplasm theory, we have come to regard nuclear and extra-nuclear protoplasms as quite unlike one another, colloidal changes in the two are quite probably influenced by the same factors.

From microdissection experiments Chambers (5, 6, 7) concludes that astral rays are fluid and the regions between them are in the gel state. He regards the rays as channels along which fluids move into the centrosphere from all parts of the cytoplasm. Cross sections of polar caps and spindle regions of *Spirogyra setiformis* show two kinds of materials, one appearing as circular areas, which do not stain, and the other occupying the spaces between these circular areas, which stains readily. It is possible that these two kinds of materials may correspond to Chambers's fluids and gels of the aster. The gels, which in ordinary cytoplasm are fairly evenly distributed among the sols, may have condensed in such a manner as to produce the appearance seen in cross sections of striated areas in *S. setiformis*. They exist in regions which are undergoing rapid increase in size which must be due to a rapid influx of fluids. This also fits Chambers's theory with respect to the function of the component parts of the achromatic figure.

Miss Ma (13) has observed the presence of a fibrous appearance in the division of the large single plastids in the spore mother cells of *Physcomitrium*. These apparent fibers are seen in the thin, flat isthmus joining the two halves of rapidly dividing plastids. They are identical in texture and

staining reaction with fibers in heterotypic meta- and anaphase figures in adjacent mother cells. The division of these colloidal masses has been regarded as the simplest fission, involving no accurate distribution of materials as in nuclear division, nor the formation of a limiting membrane. Yet this moss plastid possesses these important "fibers," at least during a part of its division, and it is probable that other rapidly dividing plastids will be found to have similar structural features. A phenomenon common to both must be operative in the division of these plastids and the division of nuclei and cells. Since it is quite obvious that the flow of the colloidal material of the plastid must be responsible for its separation into two parts it seems reasonable to associate this flow with the fibrous appearance in the isthmus of a dividing plastid. Protoplasmic flow has long been associated with the phenomena of nuclear division and while it is probably not the sole agency at work, it is undoubtedly present during this period of active movement of the protoplasm.

Reference has been made to the work of Tangl (31) in which attention is directed to the vacuole-like structures between the chromatic masses of the anaphases and telophases in *Spirogyra*, to which he gave the name "Verbindungsschlauch." He believed that the membrane of this vacuole was derived to a large extent from the flanks of the nuclear membrane. Strasburger (30) disagreed with him on this point and regarded the membrane as arising wholly from cytoplasm. Strasburger also believed that the Verbindungsschlauch pushed the chromatic masses and daughter nuclei apart and that it was also of importance in the formation of the cell plate. He shows the tubular nature of the structure in the middle stages of its development especially clearly in his *S. polytaeniata* and regards it to be present in *S. nitida* and *S. majuscula* which he had studied earlier. There seems little doubt that this hollow phragmoplast arises from a vacuolate connecting strand of protoplasm and not from disconnected strands of protoplasm.

From the interpretations of the data obtained, the achromatic figure in *Spirogyra setiformis* arises as a series of colloidal changes, first showing as structural changes in the cytoplasm at the poles of the nucleus. These changes seem to comprise rearrangements of the constituents of the cytoplasm in such a manner as to produce long cylindrical areas free from stainable material, or, at least, made up of material staining with considerable difficulty. These areas are surrounded by finely granular material which stains readily. These changes arising in the cytoplasm extend through the nuclear membrane into the nucleus. There is no evidence of the passage of actual cytoplasmic material into the nuclear cavity.

In the later prophases the arrangement of the cytoplasmic constituents is again changed and the materials are redistributed to produce the appearance of the ordinary alveolar cytoplasm.

The development of the achromatic figure in *S. setiformis* follows in its

general details that described by Flemming (9) for certain "rundkernige" Spirogyras. His interpretation of an intra-nuclear spindle in these forms has never been favorably received by other investigators of the mitotic phenomena in the genus. In *S. setiformis* the achromatic figure at the metaphase stage is clearly surrounded by a definite membrane. Earlier stages show that the membrane is without doubt the nuclear membrane and that the polar caps have grown smaller and the final residue has merged with the cytoplasmic accumulations at the poles. That cytoplasm actually forms a part of this intra-nuclear spindle is not clear. The appearance of fibers passes from the polar cap into the nuclear cavity, and is not followed immediately by any noticeable decrease in the size of the caps. Since the nuclear membrane remains intact at all times, it seems reasonable to conclude that, if any parts of the cytoplasm enter the nuclear cavity, it must be the more fluid parts and not the solider granular constituents. The view formerly held that there is a very marked difference in the origin of intra- and extra-nuclear spindles is gradually giving way to the idea that the difference between the two is a matter of the time of the disappearance of the nuclear membrane. This seems to be a tacit admission that the source of the protoplasm in the achromatic figure is not hard and fast and that in some cases it may be cytoplasmic and in others of nuclear origin and in others a part of both. In other species of *Spirogyra* this intra-nuclear nature of the achromatic figure is either very inconspicuous or not recognizable. The achromatic figure is apparently formed from the nucleus plus the polar caps, and the polar limits of the polar caps become the polar limits of the achromatic figure.

The formation of the cell plate is in general as it is in the higher plants —by the condensation of the protoplasm, especially the solider portions (the gels), in the equatorial region. This seems to be brought about by the movement of the more stainable material along the apparent fibers of the phragmoplast and the condensation to form the cell plate. This process is probably as definite as it is in the higher plants.

This type of cell plate formation from a hollow phragmoplast has been shown repeatedly for *Spirogyra* and has been described and figured for *Zygnema* (8), *Mougeotia* (22), and *Closterium* (12), as well as for *Cladophora* (3). It seems to be general for the Conjugatae. In *Oedogonium* (18, 33), on the other hand, the cell plate develops from an eccentrically located disc which extends to the lateral wall to form the completed septum. In *Scenedesmus* (25), *Pediastrum* (26), and *Tetraedron* (27), Smith figured the dividing cells as separating first in the interior of the cell, apparently in many cases before the cell plate is apparent at the lateral wall. This has been also shown by the author for *Tetraspora* (14). Miss Carter (4) finds the division membrane of *Ulva* arising at one side of the cell between the daughter nuclei and growing across the cell. None of these examples show any evidence of the growth of the cell plate from the periphery to the center

of the cell. It is very probable that in these last mentioned algae the cell plate is formed essentially as in the higher plants, developing from the interior of the cell to the periphery. Miss Goldstein (10) has shown that during cytokinesis in cells of tobacco which have large central vacuo'es, the partition often begins close to one side of the cell and grows across the vacuolate area, new phragmoplast fibers being formed ahead of the developing membrane. This of course is necessitated by the large size of the vacuole and the consequent sparsity of the cytoplasm. The nucleus is forced to occupy an eccentric position. Such an eccentric beginning of the wall has been mentioned as occurring in *Oedogonium* and *Ulva*. The cytokinesis in cambium cells is known to take place in much the same manner. In *S. setiformis* beginning cytokinesis is not recognizable until the mid-nuclear vacuole, the "Verbindungsschlauch," has enlarged so that it has come into contact with the lateral wall, thus augmenting the amount of cytoplasm at the point of contact between the cytoplasm at the periphery and that surrounding the hollow phragmoplast.

The Conjugatae, in general, are peculiar in having the nucleus and the achromatic figure suspended in the center of a large vacuole. The scantiness of the cytoplasm in this region may help to explain why the members of this group have come to initiate cell plate formation at the periphery of the cell where the cytoplasm is more abundant.

Referring to the theory of fibrillar contractility in connection with nuclear division, Wilson (34) says "the whole hypothesis gradually lost ground and is now almost abandoned despite vigorous attempts to modify and improve it." Reference has already been made to the theory of Meves (16) who advanced the hypothesis that the fibers are pushing organs. Evidences of the remarkable vitality of the hypothesis that fibers draw or guide chromosomes to the poles are seen in recent literature. Recent genetical and cytological work of Muller and Painter (20, 21) on cells of *Drosophila* shows that, as a result of exposure to X-rays, chromosomes may become separated into two parts. That part of the chromosome having no connection with the spindle fiber may become attached to another chromosome or it may not. In the latter case it is lost and no longer plays any rôle in the genetics of subsequent generations. If connected with another chromosome it participates in the genetical phenomena but with linkage relations necessarily changed. This is interpreted as showing that the spindle fibers are instrumental and necessary in bringing the chromosomes to the poles of the cell.

Trankowsky (32) has described and figured special bodies on the chromosomes of *Bellevallia* (*Hyacinthis romanus*), *Crepis*, and *Naias* at the regions of the attachment of the spindle fibers. According to his account they are present in the late prophases before the spindle fibers show attachment to the chromosomes. Since the stain has to be very weak in order to differentiate these bodies from the rest of the chromosome, the spindle fibers

are stained very slightly or not at all. The bodies are present at the bends of the chromosomes at the regions where fibers would be expected to be seen.

Summary

The achromatic figure is apparently due to colloidal changes in the protoplasm, arising in the cytoplasm and moving into the nucleus. Since the nuclear membrane remains intact during these changes any material interchange between the nucleus and the cytoplasm must be of the most fluid materials.

The fibrous appearance of the achromatic figure is due to aggregations of protoplasmic material which stains readily, lying at the points of junction of several elongated cylindrical areas which do not stain.

Because of the disconnected condition of the nucleolar and chromatic materials of the equatorial plate its movement cannot be due to the action of fibers, for a fiber would be needed for every loose particle. The movement is such as could be expected from the flow of the materials.

The phragmoplast, though a hollow structure, forms the cell plate in essentially the same manner as phragmoplasts in the cells of the higher plants.

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EXPLANATION OF PLATES

PLATE LIX

Figures were all made with camera lucida and the magnification is × 1050, except where otherwise stated.

FIG. 1. Early prophase showing beginnings of striated condition at poles. Nucleole unchanged.

FIG. 2. Slightly later than figure 1. Inner striated caps not yet clearly separated from the unstriated cytoplasm.

FIG. 3. Striated polar caps well differentiated. Nucleolus very large and uneven.

FIG. 4. Fibrous condition on the interior of the nucleus.

FIG. 5. Cross section of polar cap at a stage probably slightly later than in figure 3, showing spongy appearance from this view.

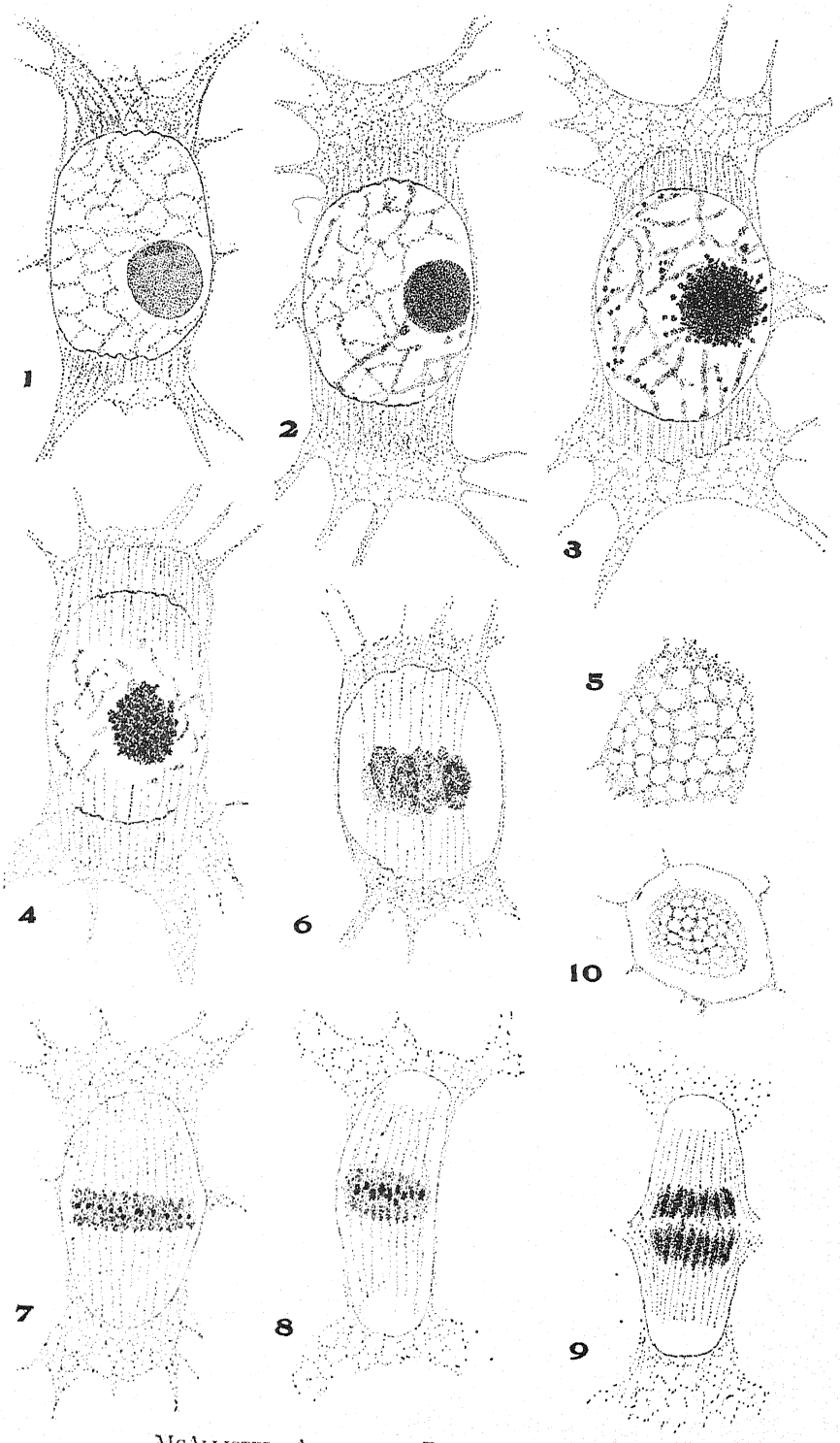
FIG. 6. Chromatic material accumulating on the equatorial region of the nucleus. Fibrous condition has disappeared from cytoplasmic caps.

FIG. 7. Metaphase showing two kinds of material making up the equatorial plate. Nuclear membrane still intact.

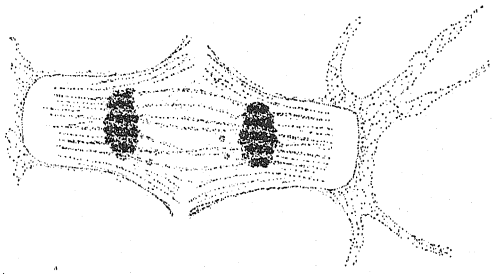
FIG. 8. Slight later metaphase showing clear spaces in the poles of the figure.

FIG. 9. Early anaphase stage showing rupturing of nuclear membrane at the equator. Chromatic material in diffuse rod-like arrangement.

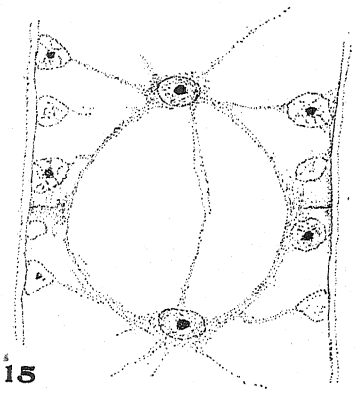
FIG. 10. Transverse section of fibrous region of anaphase figure at a stage slightly earlier than that shown in figure 12.



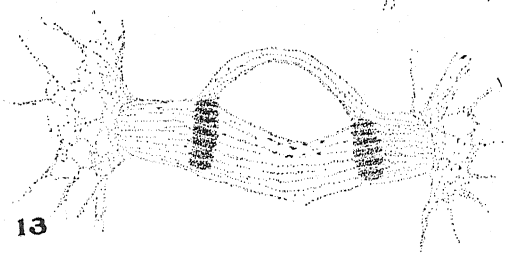
McALLISTER: ACHROMATIC FIGURE IN SPIROGYRA



11



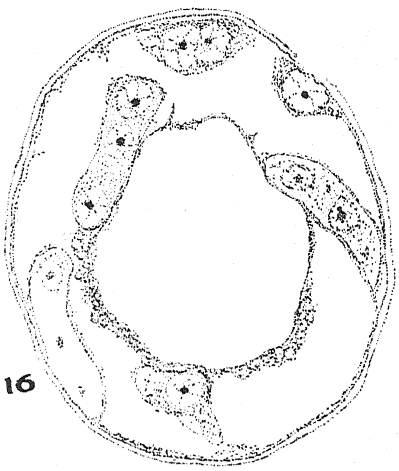
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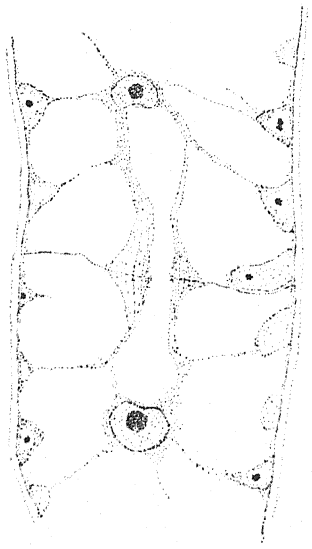
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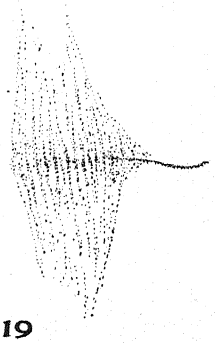
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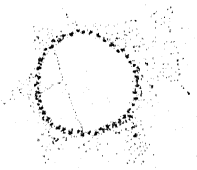
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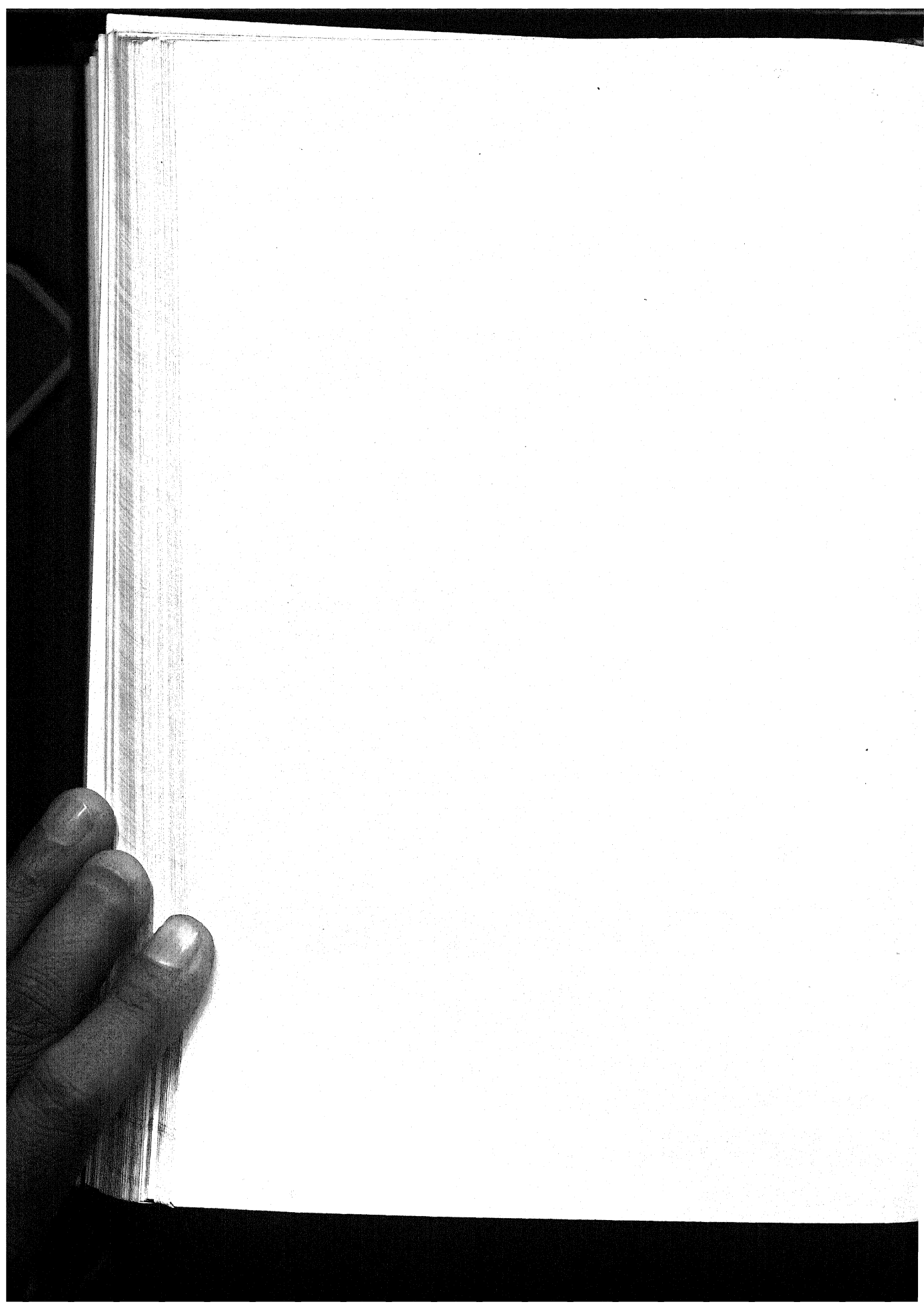


PLATE LX

FIG. 11. Later anaphase.

FIG. 12. Transverse section of anaphase figure slightly older than that shown in figure 11, taken between two chromatic masses.

FIG. 13. Later anaphase showing a vacuolar swelling in the central spindle.

FIG. 14. Early telophase showing larger central vacuole in spindle.

FIG. 15. Telophase spindle showing early stage in the development of diaphragm. A large vacuole lies between the daughter nuclei.

FIG. 16. Transverse section of telophase slightly later than that of figure 15. Taken midway between the daughter nuclei. Shows the continuous membrane surrounding the vacuole of the "central spindle."

FIG. 17. Late telophase with diaphragm nearly completed. Shows depressions on nuclei where in contact with the vacuole. × 850.

FIG. 18. Transverse section of vacuole midway between diaphragm and nucleus. Approximately the same age as in figure 17.

FIG. 19. A portion of the "central spindle" from a telophase similar to that in figure 17 showing membrane formation. × 2000.

STUDIES ON THE MORPHOLOGY OF THE ONAGRACEAE VI. *ANOGRA PALLIDA*

DONALD A. JOHANSEN

(Received for publication June 23, 1931)

The genus *Anogra*, as its relationships are at present understood, stands somewhat isolated in the family Onagraceae. Its closest affinities are doubtless with *Hartmannia* (2) and *Raimannia*, but *Anogra* seems to have become separated from related genera so long ago that many profound changes have occurred during the intervening period, which changes have made it somewhat difficult to recognize other than superficial resemblances. *Hartmannia* and *Raimannia* in all likelihood originated in the high plateau region of central Mexico and thence at least one group of species in each genus spread northward. Later expanding into what is now southern California and thence as far north as Alberta, some species inevitably entered the arid regions of this stretch of territory. It is from such species that *Anogra* perhaps originated.

Nearly all the species of *Anogra* are today restricted to desert or at least to very arid regions. It is well known that adaptation to a desert environment invariably requires structural modifications in plants, and *Anogra* is no exception to that principle. This fact is revealed primarily in a greatly decreased ability to produce viable seeds; in some cases no seeds at all are formed, the perennial plant reproducing itself by means of offshoots at the ends of subterranean stolons. One species that exhibits both phenomena clearly is *Anogra pallida* (Lindl.) Britton, which has a rather wide geographical range for a species of *Anogra*, this being from southern Arizona through California northwards to the eastern part of Washington and adjacent Idaho.

MATERIAL

The material on which this study is based was secured from a number of plants sent me in the spring of 1929 by Mr. D. H. Snowberger of Payette, Idaho, a keen and intelligent observer who kindly furnished valuable information concerning the species. The plants were secured from the sand-hills near Payette. Mr. Snowberger once secured some seeds from plants growing in his garden, but states that it is extremely rare to find them. The capsules are very hard, and have never been observed in dehiscence. Seedlings have never been found in nature, and none has been seen in my own garden.

OVARY AND OVULES

There is nothing atypical in the structure of the ovary, save that protuberances of a fairly large size, hypodermal in origin, are often to be

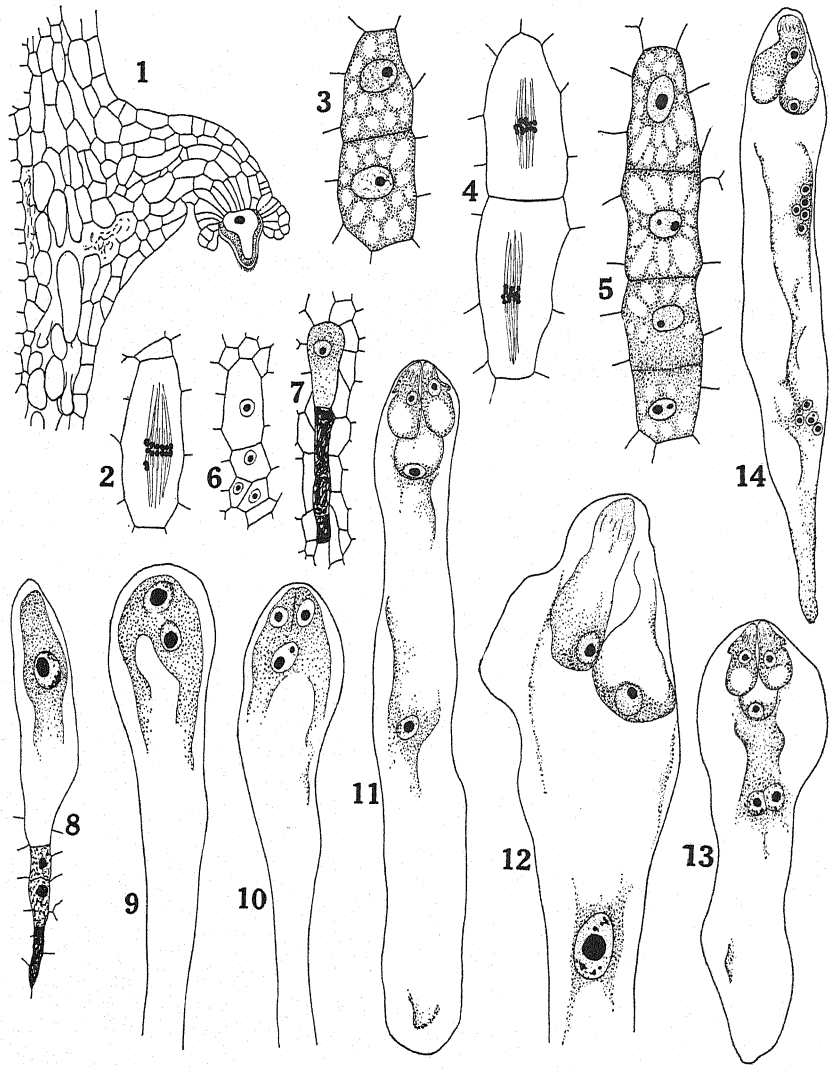
found at various points on each ovary. In a few instances there is some evidence of a vascular supply to the structure. If these structures are relics, it might indicate that the ovary was leaf-bearing at one time, exactly as the ovaries of *Gongylocarpus* and *Burragea* are leafy. In other cases, as in text figure 1, the protuberance assumes the appearance of some sort of apparently degenerated epidermal appendage, such as a trichome.

When one compares different conceptions of the number of rows of ovules and the arrangement of the ovules within each loculus of the ovary, two contradictory statements are encountered. The older conception, perhaps best represented by Small's (6) treatment, calls for the presence of two rows of ovules and seeds, while the more modern delimitation of the genus, as outlined by Sprague and Riley (7), specifies a single series of ovules. The latter claim, with which the present series of morphological investigations is in full agreement, is the more accurate one. In *Oenothera* proper, the ovules are horizontal and two- to four-seriate; in *Raimannia* they are ascending and biseriate; in *Anogra*, vertical and quasi-uniseriate; in *Hartmannia*, numerous and clustered on slender funiculi. In respect to the number of rows of ovules and their orientation, each genus is quite distinct and these characters are of definite, if ignored, taxonomic value. The present paper, however, is hardly one in which to enter into a discussion of the phylogenetic significance of these characters.

The ovules of *Anogra* are not arranged in a strictly uniseriate order. In nearly every loculus are to be found one to several extra ovules located to one side of the normal series and somewhat between two successive ovules. A few ovaries may even be found which possess two complete rows of ovules in each loculus. These supernumerary ovules, doubtless either a survival from, or a throwback to, the ancestral forms, may become so numerous in some species that the ovules have actually been described as being biseriate. Concerning such species, I should prefer to withhold judgment as to whether they properly belong in *Anogra* or not, until such time as living plants become available for study.

The orientation of the ovule with respect to the position of the micropyle, whether directed towards the base of the ovary (normal position) or upwards, is somewhat irregular. In *A. deltoides* and in *A. trichocalyx* the micropylar apices of the ovules in each half of the ovary are regularly directed towards the center of the ovary—truly an unusual circumstance. This character is apparently specific for these two species. In *A. pallida*, the majority of the ovules are normally oriented, but a varying number in each loculus is directed oppositely. The position of the ovule in this species, at any rate, is of no particular significance since fertilization occurs so rarely that for all practical purposes it may be considered as never taking place.

In a small proportion of the ovaries, the nucellus of every ovule has completely collapsed; the method by which this is brought about is that described for Type *C* sterile ovules in *Taraxia ovata* (3).



TEXT FIGS. 1–14. 1. Protuberance on ovary, showing resemblance to a trichome; median longitudinal section. × 96. 2. Anaphase of first division in megasporocyte. × 1000. 3. Secondary megasporocytes. × 1000. 4. Anaphase of second division in megasporocytes. × 1000. 5. The quartet, just completed. × 1000. 6. Quartet in which lower two megaspores are irregularly arranged. × 380. 7. Functioning megaspore of a weakly developed quartet. × 380. 8. Same, well developed. × 380. 9. Binucleate megagametophyte. × 380. 10. Trinucleate megagametophyte. Lower nucleus has not yet divided. Synergid wall formation beginning. × 380. 11. Well-organized mature megagametophyte. × 180. 12. Beginning of partial disorganization of egg cell and synergids (only one shown) preparatory to multipartitioning of polar nucleus. × 380. 13. First amitosis of polar nucleus. × 180. 14. One section of embryo sac showing ten of the thirty-four polar nuclei. × 180.

Megagametogenesis

The development of the quartet follows the scheme typical for the entire family (text figs. 2–7). Meiosis has been studied to some extent in this species, in order to ascertain whether meiotic irregularities might conceivably underlie the phenomena which later occur in the embryo sac. The reduction divisions were found to be perfectly regular and no variations were noted in the haploid number of chromosomes, which is seven. Text figure 2 illustrates an early anaphase of the first division, and text figure 4 the same stage in the second division. The stages in meiosis preceding the first anaphase are not as clear as they should be, owing to the difficulty of properly fixing such a deeply embedded cell as the megasporocyte. In two fairly clear diaphase stages it was noticed that one pair of chromosomes were arranged in a closed ring and the remaining twelve elements were disposed in the form of a chain, exactly as in *Oenothera lamarckiana* and in other plants. However, the appearance of most of the metaphase plates suggests that in a normal diaphase all chromosomes exist as single homologous pairs. The appearance of the chain arrangement once in an extremely high number of cases is also reported elsewhere for *Clarkia elegans* (1).

The micropylar megaspore of the linear row of four composing the quartet is always the functional one (text figs. 7, 8). The remaining three megaspores degenerate so slowly that their remains can be perceived long after the megagametophyte has attained maturity. Occasionally the two lower megaspores are arranged side by side instead of one above the other (text fig. 6). The nuclei of these lower two megaspores are often observed to be entirely destitute of chromatin, consisting only of an empty shell. Sometimes secondary megasporocytes or quartets are found to be more or less completely degenerated; but in spite of this, it is extremely rare to find an uncollapsed ovule in which there is no megagametophyte.

The mature megagametophyte possesses the typical onagrad structure, but in comparison with other species is considerably longer in proportion to its breadth (text figs. 11–13). The nucellus is attacked to only a very slight extent. The synergids each have a deep indentation and a well developed so-called filiform apparatus, but no exudations have been noted

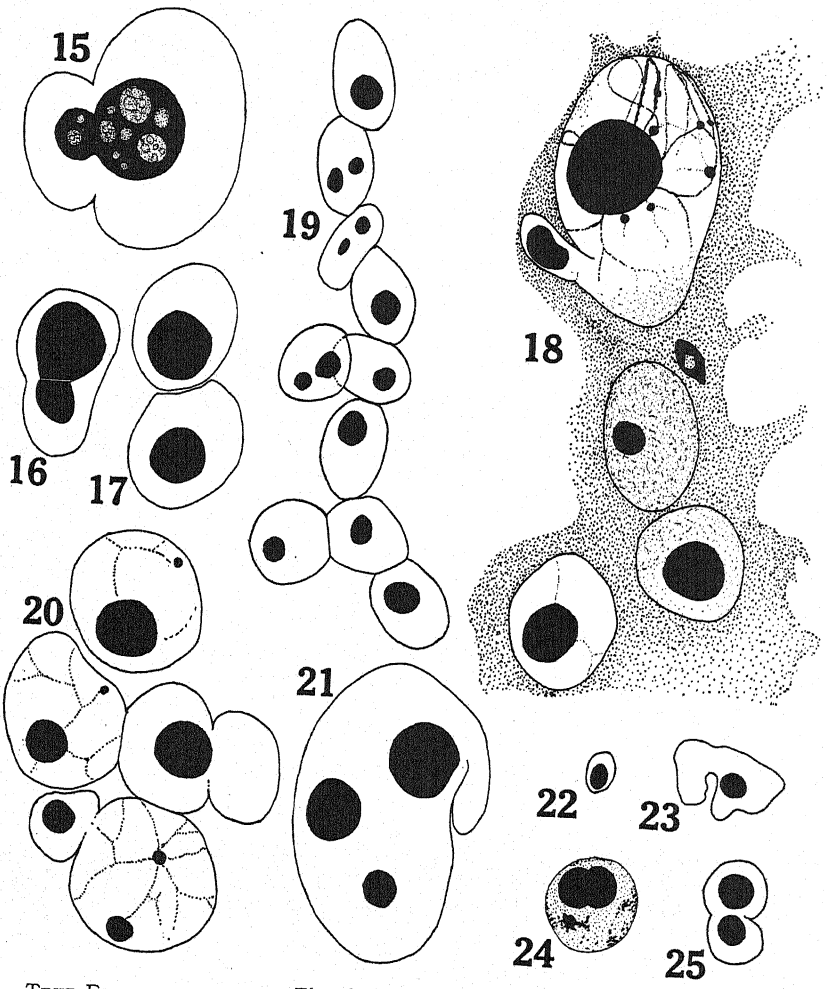
Phenomena in the Embryo Sac

Fertilization ordinarily does not occur in *A. pallida*. No traces of pollen tubes have been found anywhere in the stigma or ovary. An examination of the microspores reveals that although their morphology is essentially normal, the protoplast is very small and nearly completely shrunken. The pollen grains seem wholly incapable of germination, except perhaps under exceptionally favorable conditions which might occur only once in about seven years or more.

In most onagrads, as a rule, the embryo sac degenerates within a short

time if fertilization is not effected. In *A. pallida* this does not happen; instead, a most unusual phenomenon occurs. A description of a similar condition in any other plant is not known to the writer. The phenomenon does not take place in every ovule but only in a varying proportion of the total number in each ovary. The percentage may in some cases reach ninety, but is generally around twenty.

The polar nucleus in *A. pallida* evidently possesses an unusual vitality, and in addition has the capacity for growth and indefinite division. In other onagrads the polar nucleus is generally the first to disappear during the



TEXT FIGS. 15–25. 15–17. First fission of polar nucleus. 18. Young group of nuclei. Parent nucleus is uppermost one. 19. Older group of actively dividing nuclei. 20. Group from very actively partitioning mass of nuclei. 21. Largest, and 22, smallest, nuclei found. Notice split in former. 23. Nucleus with irregular contour; perhaps result of malnutrition. 24–25. Beginning of fission in synergid nucleus. All × 1250.

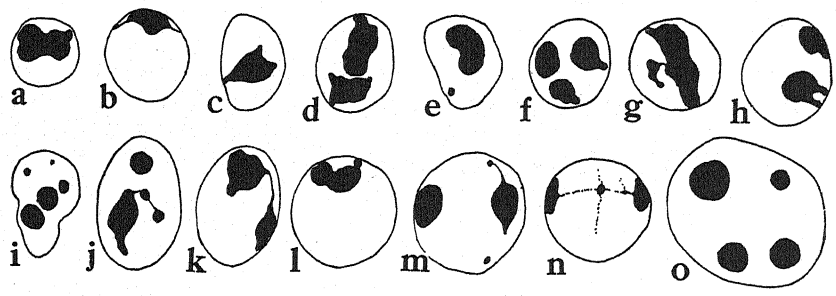
degeneration of the megagametophyte; *Zauschneria latifolia* (5) was recently reported as an exception and with this species should not be included *A. pallida*.

The phenomenon for convenience may be described as multipartitioning of the polar nucleus (text figs. 13–21, 24–27). The process is wholly an amitotic one, no mitoses having ever been seen. Fission is accomplished by the division of the nucleolus simultaneously with, or shortly followed by, the cleavage of the nucleoplasm. The first division of the polar nucleus has been observed several times; text figures 13 and 15–17 show typical cases selected at random. The nucleolus initiates amitosis and the nucleus almost immediately thereafter displays a bulbous projection at one side. In the middle of the fission process the nucleolus acquires, for a very short time, the appearance of having a bubbly-frothy structure. Ordinarily this condition could readily be attributed to faulty fixation but in the plant under study it doubtless is to be considered a good indicator of the abnormality of the polar nucleus since it is not met with at any other time. After the first fission, the relation of the two resultant nuclei is that of parent and offspring, not that of sisters. The parent nucleus can be recognized as such, in most of the ovules in which multipartitioning occurs, until as many as thirty-five offspring nuclei are to be counted in the embryo sac (*e.g.*, text fig. 18, uppermost nucleus). The first offspring nucleus is at first very much smaller than the parent (text figs. 15, 18, 27 *b*, *d*, *f*, *g*, *t*, *u*) but rapidly increases in bulk, its nucleolus maintaining a corresponding and proportionate increase, until the two are practically equal by the time separation finally ensues (text figs. 17, 27 *g*, *h*, *k*, *m*). The nuclei from all further fissions vary greatly between different ovules, even if the latter are adjacent to one another, but in general they tend to maintain the size of the first daughter nucleus for some time. This is about the opposite of what happens in other onagrads after the polar nucleus is fertilized: in these plants the successive generations of "endosperm" nuclei gradually decrease in size until finally there remain mere vestiges of chromatin incapable of further divisions. In other words, the "endosperm" nuclei lack the capacity for growth, and it seems plausible enough that this may eventuate from the fact that increase in number of "endosperm" nuclei is through mitosis. In other megagametophytic structures, *e.g.*, antipodals, nuclei which arise amitotically are known to increase in bulk. In fact, in *A. pallida*, in none but the most poorly nourished sacs is there much diminishing in bulk of the successive generations of nuclei. The extreme variations in size are illustrated by the nuclei in text figures 21 and 22, respectively.

The rate at which amitosis proceeds does not seem to be governed to any great extent. The amount of nourishment available is apparently the principal limiting factor. A second factor is the age of the megagametophyte at the time the first partitioning of the polar nucleus occurs; the

later the time, the fewer the divisions, but, strangely enough, the larger the offspring nuclei.

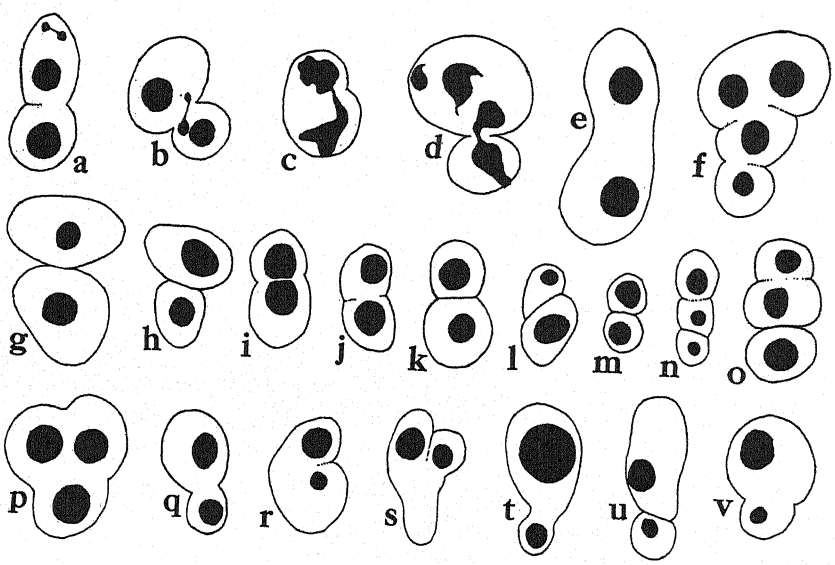
The contour of the nuclei ranges from spheroid to ellipsoid, depending somewhat on the metabolic rate. That is, the lower the rate, or the nearer the nucleus is to the resting condition, the more spherical it becomes. Some few nuclei, in poorly nourished ovules, have an irregular contour



TEXT FIG. 26, *a–o*. Group of nuclei more or less at rest, to show diversity in appearance and size. × 1250.

(text fig. 23), as have most nuclei when disintegration finally sets in (text fig. 28).

The number of nuclei, exclusive of egg cell and recognizable synergid nuclei, in twenty-six ovules chosen entirely at random from twenty ovaries, were counted. The numbers follow: 3, 4 (thrice), 6, 8, 14, 15 (thrice), 16 (twice), 18, 19, 21, 22, 23, 30 (twice), 34 (twice), 35, 37, 74, 96, 140. The



TEXT FIG. 27, *a–v*. Group of nuclei at various stages of amitosis. × 1250.

largest number counted is therefore one hundred and forty nuclei, which were in a large and well nourished ovule. Text figure 19 shows a group of actively dividing nuclei from the same ovule, and by comparing this group with the group in text figure 20, in which the total number is eight, it can be seen that they have decreased considerably in bulk, but are still actively dividing. It is impossible to say what the maximum number might be, since so many variable factors are present.

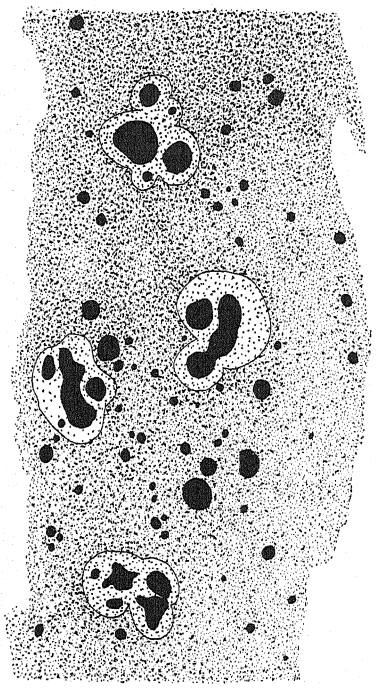
Text figure 26, *a–o*, shows a group of "resting" nuclei especially selected from many ovules to show the great variation in amount of chromatin and behavior of the nucleolus or nucleoli. The highest number of nucleoli observed in any one nucleus is five. The nucleus does not always split after the fission of the nucleolus; by comparing the nuclei in text figure 26 with those in text figure 27, it will be seen that the nucleolus is most active when the nucleus is otherwise apparently at rest.

In text figure 27, *a–v*, are shown twenty-two nuclei in various stages of division. The great ability of even the smallest nuclei to divide is shown in the nuclei at *m* and *n*. Fission of the nucleus may be entirely independent of the division of the nucleoli (text fig. 27 *e*, *q*, *t*, *v*) or splitting of the two bodies may be simultaneous (text fig. 27 *b*, *c*, *d*). In many nuclei may be seen a secondary nucleolus, usually much smaller than what is apparently the principal nucleolus, which is in amitosis at the time the nucleus is dividing. Occasionally it divides within one daughter nucleus (text fig. 27 *a*, *d*), or may rarely divide in the zone of cleavage in such a manner that each daughter nucleus receives part of it (text fig. 27*b*). The method of cleavage is well shown in the nuclei in text figure 27, and seems to be essentially similar to the process of splitting in pollen tetrads; text figure 27*p*, for instance, closely resembles a trinucleate microsporocyte at the beginning of the process.

A very few instances were discovered where the nucleus of one of the two synergids may initiate divisions in a manner exactly comparable to the behavior of the polar nucleus. The first stages are shown in text figures 24 and 25. Older stages, not often found, do not differ much in appearance from the polar nuclei figures. The synergid nuclei do not become reproduced to any great extent; it is doubtful if the total number in any one embryo sac exceeds twenty. The cytoplasm in which these nuclei lie is of very small extent and is quite dense. The groups of synergid nuclei are the first to show signs of disintegration; an advanced stage in the dissolution of the nuclei is shown in text figure 28.

No information is available as to what finally happens to the offspring of the multipartitioned polar nucleus; the oldest stages found were in ovules which, if they had been fertilized, would contain embryos in the final stages of cotyledon growth; hence it can only be conjectured that the nuclei eventually become disorganized upon the desiccation of the capsule and thus die a more or less natural death.

A. pallida closely resembles *Stenosiphon linifolium* (4) in one respect, namely, in that no apomictic or abnormal embryonal structures of any nature have ever been encountered in either species. Considering the tendency of some species, *e.g.*, *Zauschneria latifolia* and *Taraxia ovata*, to produce irregular embryos freely, this is certainly a fact which should be given due consideration.



TEXT FIG. 28. Degeneration and disorganization of an old group of synergid nuclei. × 1250.

DISCUSSION

The observations recorded above do not provide any information of immediate practical value in ascertaining the phylogenetic position or relationships of the genus *Anogra*. Multipartitioning of the polar nucleus is not known in any other species of the Onagraceae so far studied, hence the exact significance of the phenomenon is a problem whose solution can come only at some future time.

SUMMARY

1. *Anogra pallida* is a nearly completely sterile species, reproducing by means of offshoots borne at the ends of subterranean stolons.

2. The ovules are disposed in a vertically quasi-uniseriate order; this is a generic character of importance.

3. Meiosis and development of the quartet are regular.

4. The micropylar megaspore is the functional one; the mature megagametophyte possesses the typical tetranucleate onagrad structure.

5. Fertilization occurs so rarely that it may be considered as non-existent.

6. In an average of twenty percent of the ovules in any particular ovary, the polar nucleus undergoes multipartitioning. The daughter nuclei possess the capacity for growth and repeated division. The number of nuclei thus arising amitotically may reach 140 in well-nourished megagametophytes.

7. The nucleus from one of the synergids may likewise reproduce itself by fission, but the number of free nuclei does not exceed twenty.

8. No apomictic or other embryonal structures could be found in the more than 16,000 ovules examined microscopically.

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INVESTIGATIONS ON THE MORPHOLOGY AND COMPARATIVE HISTORY OF DEVELOPMENT OF FOLIAR ORGANS II. CATAPHYLL AND FOLIAGE LEAF FORM AND ORGANIZATION IN THE BLACK HICKORY (*CARYA BUCKLEYI* VAR. *ARKANSANA*) [1]

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INTRODUCTION

The first paper in the series (Foster, 1929) presented a detailed account of the morphology and ontogeny of the foliage leaves and cataphylls in *Aesculus Hippocastanum* L. It was found that the bud scales proper consist of (1) a more or less distinctly winged vaginate portion, which is developed by active marginal growth from the basal region of the foliar primordium and (2) an apical group of distinct but rudimentary leaflet primordia, which were shown to be homologous with the leaflets of the foliage leaf. In spite of the obviously segmented character of the cataphylls in this species, these structures were found to exhibit a divergent ontogeny as compared with the foliage leaf and, for this reason, are not regarded as "arrested formations" in the sense with which Goebel (1884, p. 251) uses this expression. It is of considerable importance to my conclusion to note that Schüepp (1929), in a recent paper, has reported that the inner bud scales of *Acer Pseudoplatanus* L., which are of a type similar to that of *Aesculus Hippocastanum* L., diverge ontogenetically from the foliage leaf at an extremely early stage, perhaps even before the differentiation of the lamina and leaf base has occurred. It seems only proper to conclude that further intensive studies, of the type made by Schüepp and the writer, are urgently needed and may be expected to place the problem of cataphyll morphology in a new and more hopeful position (cf. Foster, 1931).

Since the publication of my investigations on *Aesculus*, I have made a similar type of study on the black hickory (*Carya Buckleyi* var. *arkansana* Sarg.). My attention was originally attracted to this species by the fact that the large, inner foliaceous bud scales, while somewhat similar in general form to those of the horsechestnut, showed no evidence of discrete lamina primordia although the foliage leaves are segmented and of the type designated as imparipinnate. It was evident, therefore, that the relationship between form and organization (*i.e.*, morphology) is of a different order in

[1] Contribution from the Botanical Laboratory of the University of Oklahoma, N.S. No. 11.

the cataphylls of the black hickory than in those of the horsechestnut and hence deserved serious investigation, from both a comparative as well as a developmental standpoint.

In the present paper, the results of a detailed comparative study of the cataphylls and foliage leaves in the black hickory are presented. Certain conclusions as to the morphology of the cataphylls will be drawn from evidence furnished by the form and venation of these organs. The validity of these conclusions will then be further examined in the light of the comparative ontogeny of the cataphyll and foliage leaf which will form the subject of the third paper of this series.

Materials and Methods

The material used in this investigation was obtained from trees growing about seven miles east of Norman (Cleveland County), Oklahoma. Both fruiting and vegetative shoots from one of the trees studied have been deposited in the herbarium of the Arnold Arboretum, Jamaica Plain, Massachusetts, and I am indebted to the curator, Doctor Alfred Rehder, for determining this material as *Carya Buckleyi* var. *arkansana* Sarg. General information as to the botanical characteristics and range of this tree are given by Sargent (1922, pp. 198–199); the common name "black hickory," has been adopted from Mattoon, Phillips, and Gibbs (1930, p. 34).

Frequent collections of material were made during the "rest" and growing periods of 1928–1931 and laboratory studies were supplemented, whenever possible, by field observations. Both resting and expanding buds were preserved for study in a five percent solution of formalin, which not only toughens the material for dissecting purposes but likewise renders the venation of the inner cataphylls and foliage leaves fairly apparent. Better results, as regards clearing the scales for venation studies, were secured by the following method: living material was first immersed for a short time in boiling water and then gently blanched in hot 95 percent alcohol; after all the visible chlorophyll had been extracted, the cataphylls were placed in a concentrated solution of chloral hydrate and allowed to remain there until their venation was easily visible.

All detailed observations were made with a Spencer dissecting microscope at magnifications of 20.7 to 61.2 diameters.

The General Organization of the Shoot System

The shoot system of the black hickory is definitely of the monopodial racemose type[2] with active elongation proceeding annually from the terminal buds of the main shoots and relatively slower apical extension occurring in the "dwarf" or "spur" branches which usually arise during the second year of life of a main shoot from its axillary buds.

[2] Using the terminology of Domin (1923, p. 4), the branching in the black hickory is of the "pleuroblastic racemose" type.

On both the long and spur shoots, a periodic alternation of cataphylls and foliage leaves occurs which is only normally broken by the appearance of the terminal female inflorescence (Pl. LXIII, fig. 2). The staminate catkins, which appear in threes on a common peduncle, normally arise in the axils of the uppermost cataphylls of the long or spur shoots; upon falling, they leave characteristic oval scars on the twigs (Pl. LXIII, fig. 1). I have found staminate inflorescences occasionally appearing from the axils of the lowermost foliage leaves of the annual shoot but this appears to be an infrequent condition.

The black hickory, like all species of *Carya* (cf. Trelease, 1896, Pls. 13, 14, 15), usually shows, at least on vigorous long shoots, one or more serially arranged accessory buds at a node in addition to the main axillary bud (Pl. LXIII, figs. 1 and 2). Although the biological relationship of these accessory buds has not been investigated, they appear to be "reserve buds" which only develop when the shoot from which they have arisen is injured or abnormally stimulated in some way.[3]

Structure of the Terminal Bud

External Features

The terminal buds of the black hickory are ovate-acute or ovate acuminate in form, from 6.5–9 mm. in length, and possess 2–3 exposed, reddish-brown bud scales which are covered with scattered pubescence and numerous dry, yellowish-white colleters. The shape of the terminal bud is in part at least determined by the behavior of the two or three outermost cataphylls. These organs (Pl. LXI, figs. 1–3, and Pl. LXIII, fig. 2), which will be described in detail later, are distinguished from the inner scales by their prominent acuminate tips, and when they are present during the winter, impart an acuminate form to the bud. Very often, however, one or more of these outer bud scales are deciduous during the autumn or winter and then the cowl-shaped exposed scales give the bud a more bluntly ovate form (Pl. LXIII, fig. 1).[4]

Scale, Foliage Leaf, and Flower Content

The number of cataphylls and foliage leaves present in a terminal bud varies within certain limits which are determined (*a*) by the well-known "individuality" of the buds in a complex shoot system and (*b*) by the type of bud with which one is concerned. It is with the latter factor that I shall deal.

The terminal leaf bud of long shoots consists of 9–11 cataphylls, 5–11

[3] In this paper, the discussion will be confined to the organization of the terminal bud and the shoot developed from it. The structure and development of the axillary buds will be treated separately in a forthcoming paper in this series.

[4] Illick (1919, pp. 112–113) has noted the deciduous character of the outer bud scales in *Carya alba* K. Koch and *Carya glabra* Sweet, and Trelease (1896, p. 38) uses the deciduous character of the outer scales in *Carya alba* K. Koch as a diagnostic mark of this species.

foliage leaves and usually the primordia of the two outer cataphylls of the terminal bud of the next season. If one is dealing with a mixed bud, one of two conditions obtains: (1) staminate catkins, in a well-advanced stage of development are present in the axils of the innermost scales,[5] six or more foliage leaves are present and the young shoot terminates in a growing point flanked usually by two scale primordia of the next season's bud; or (2) staminate catkins are present in the axils of the inner scales, a variable number of foliage leaves are present, and the shoot terminates in the embryonic female inflorescence.

One of the outstanding features of the terminal buds of the spur shoots is the greater constancy in the number of cataphylls and foliage leaves present. The dissection of a very large number of buds, supplemented by a careful count of the bud-scale and foliage-leaf scars of successive years' growth, indicates that most frequently nine cataphylls, three to four foliage leaves, and the primordia of the two outer scales of next season's bud represent the average condition in leaf buds. The distribution of the male and female inflorescences in the mixed buds of the spur shoots appears to be essentially like that obtaining in the long shoots.

In this preliminary account of the general structure of the terminal bud in winter, two important facts must be emphasized. First, the total number of foliage leaves which is destined to appear on the new shoot is preformed and recognizable in the bud. Secondly, the initiation of the terminal bud for the following season is evident in most leaf buds. These two facts indicate the highly organized character of the resting buds in this species and indicate that here, as in the case of *Aesculus Hippocastanum*, we are dealing with a well-marked, rhythmical foliar cycle (cf. Foster 1929, pp. 444–448).

Form and Organization of Foliar Organs in the Winter Bud

The Upper Transitional Forms

The two outermost scales of the terminal bud differ in certain important respects from the inner cataphylls. In the first place, these organs usually possess distinct and prominently acuminate apices, as is shown in Plate LXI, figs. 1–3. When viewed dorsally, these outer cataphylls, which I shall now designate as the "upper transitional forms,"[6] exhibit a prominent median keel which is flanked by two thin, lateral wings; the median keel merges gradually into the acuminate pointlet (Pl. LXIII, fig. 2).

Very frequently, the outer of the two upper transitional forms shows, upon careful microscopic examination, a tiny pair of undeveloped lateral leaflet primordia which are found ventrally at the base of the apical pointlet

[5] These organs differ in no essential particulars from the inner scales of leaf buds, so that it would be of little value to refer to them as "hypsophylls."

[6] Because of their position *above* the latest developed foliage leaves and because they frequently possess rudimentary lateral leaflet primordia.

(Pl. LXI, fig. 1). In such organs it seems obvious that the prominent apical pointlet is morphologically equivalent to a terminal leaflet. This belief is further supported by the ventral groove of the pointlet (cf. Pl. LXI, figs. 1 and 2), a feature which appears in the development of the blade of the terminal leaflet of the foliage leaf.

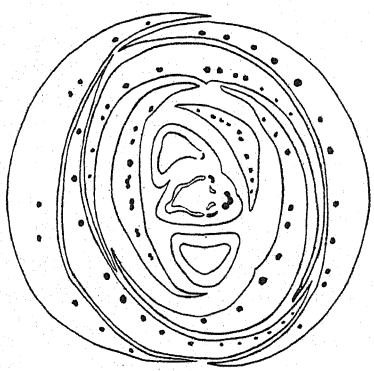
The inner of the two upper transitional forms usually shows no indication of lateral leaflet primordia but consists of a dorsally keeled, winged basal portion terminating in a prominent, ventrally grooved pointlet (Pl. LXI, fig. 3). This figure illustrates the fact that the lateral wing-like expansions of the basal portion of this organ are continuous with the ventral weals of the apical pointlet. Comparison of this organ with one such as is shown in Plate LXI, figs. 1 and 2, clearly suggests that here also the apical pointlet is the homologue of a terminal leaflet.

A study of a wide range of material indicates a great deal of variation in the size and form of the upper transitional forms and in the prominence of the apical lamina region. On the terminal buds of vigorous shoots, especially coppice shoots, the apical lamina may reach a noticeable development, while on slowly growing spur shoots, both of the upper transitional forms may be of the type shown in Plate LXI, fig. 3.

No further attempt will be made, until the ontogenetic data have been presented, to characterize morphologically the upper transitional forms. However, these organs clearly suggest the gradual appearance of certain morphogenetic relationships in the shoot which are bringing about the suppression of morphological segmentation and the *direct development* of the primordia to the bud scales proper.

The Bud Scales Proper

For purposes of general orientation, reference should be made to text figure 1, which represents a transverse section through a terminal leaf bud



TEXT FIG. 1. Transverse section through a terminal bud, showing the equitant aestivation of the bud scales and the 2/5 divergence of scales and foliage leaves. The vascular supply to the third foliage leaf is leaving the stele at the right. Vascular tissue of cataphylls and foliage leaves shown in black; stele represented in outline only.

and illustrates the more or less definite equitant aestivation of the bud scales and the striking difference in cross-sectional form between these organs and the bases of the foliage leaves. This figure likewise shows that bud scales and foliage leaves are spirally arranged with approximately a 2/5 divergence (cf. also Pl. LXIII, fig. 2). As regards their general form *in the winter bud*, the scales are somewhat vaginate, cowl-like organs and differ noticeably in their size, basal width, and extent of wings according to their position on the axis. Their organization appears relatively simple and no clearly demarcated laminar region can be distinguished.

The first scale (Pl. LXI, fig. 4), which is frequently 6 mm. in basal width and 7 mm. in height, is broadly ovate-acute in form and prominently keeled dorsally. Numerous yellowish-white, dry, colleters and scattered coarse hairs (the latter usually confined to the keel and the apical region) occur on the dorsal surface whilst the ventral surface, except in the median basal region, is covered with fine matted pubescence. The margins of the prominent lateral wings are ciliate.

The second scale (Pl. LXI, fig. 5) which is usually larger in size with a basal width and height of 7–8 mm., is broadly ovate-acute and provided with a prominent keel near the upper half of its dorsal surface. The distribution of colleters and hairs on this cataphyll is similar to that in the first scale except that the ventral pubescence is more definitely restricted to the apical region.[7]

The third scale (Pl. LXI, fig. 6) is broadly ovate, often with a basal width of 8 mm. and a height of 7 mm., and possesses prominent lateral wings, and is provided with a keel barely distinct near the apex of the dorsal surface. Hairs and colleters are distributed in essentially the same manner as in the second scale.

Certain important changes in form usually begin to appear in the fourth bud scale which averages 7 mm. in basal width and height. First of all, the wings are definitely convolute above the base of the scale and effectively embrace the rest of the bud. Secondly, a clear indication of asymmetry appears, in that the left wing of the scale (when viewed ventrally) is more constricted near the base than is the right wing. As will be shown later, this tendency towards asymmetry appears early in scale ontogeny and represents an important aspect of the general configuration of the upper cataphylls. The dorsal surfaces of the fourth and all succeeding scales of the bud present a variety of trichomes. Numerous, living colleters are thickly distributed over the whole surface and are overlaid by a dense covering of silky, yellowish-white hairs which differ structurally amongst themselves in that those occurring on the median central region of the scale (which corresponds to the dorsal keel of the outer cataphylls) are

[7] A gradual change in the quantitative and qualitative distribution of trichomes on the successive scales of a bud is a common phenomenon in Angiosperms although the "causal factors" are quite obscure are present (cf. Foster, 1929, pp. 457–458).

large and coarse whilst those found on the flanks of the cataphylls are shorter and finer in texture. The ventral surfaces of the inner scales are usually glabrous and the margins ciliate.

The steadily *decreasing basal* width of the successive bud scales is very noticeable in the fifth and sixth cataphylls. The sixth scale (Pl. LXI, fig. 7), for example, is frequently only 4 mm. in basal width and 5 mm. in height and the prominent asymmetrical constriction near its base is clearly demarcated from the noticeably convolute wings. If the wings of the fifth and sixth scales are spread out, it may be seen that these organs are more or less distinctly obovate in form, even before the expansion of the bud.

The form of the seventh scale is variable but rather frequently, as is shown in Plate LXI, fig. 8, this organ is prominently obovate-acute with an asymmetrically narrowed basal region clearly demarcated from the upper, comparatively narrowly-winged portion. Measurements indicate that this cataphyll possesses the smallest basal width of any scale in the bud (2–2.5 mm.) and that in aestivation, its wings fail to embrace completely the cluster of foliage leaves which occupies the center of the bud (Pl. LXI, fig. 9). In spite of its relatively narrow base, a transverse section of the lower portion of the seventh scale shows, like the other cataphylls, a characteristic dorsi-ventral construction with a crescentic arc of vascular bundles near its ventral surface; this arrangement of the vascular tissue is sharply contrasted with the "stelar" condition in the petioles of the foliage leaves (cf. text fig. 1).

The Foliage Leaves

The foliage leaves of the winter bud, although small in size, show a high degree of morphological segmentation which distinguishes them at once from the cataphylls (Pl. LXI, fig. 9). When the very dense covering of coarse hairs (and colleters) has been removed from the dorsal and ventral surfaces of the young foliage leaves, it may be seen that these organs consist of a well developed lamina of 5–7 leaflets, a short petiole and a broadened, more or less thick leaf base. The vernation of the blades of the leaflets is convolute-involute and an interesting relationship exists in respect to the direction of the convolutions. When viewed ventrally, the lateral leaflets on the left side show the left wing of each leaflet rolled over its right wing while the lateral leaflets on the right side have the right wing in each case overlapping the left wing.[8] The direction of imbrication of the wings of the terminal leaflet is variable; in some leaves the right wing is outermost, in others the left.

The Expanding Terminal Bud

Prior to the actual emergence of the new shoot in spring, the terminal bud increases noticeably in length and thickness. During this early period

[8] Cf. Diez (1887, pp. 67–68) and Trelease (1896, p. 32, and pl. 23, fig. 1) for similar observations on other species of *Carya*.

of bud swelling, the inner cataphylls elongate rapidly, forming a tube-like structure enclosing the cluster of enlarging foliage leaves. Usually within a week after the bud has begun to swell, the tips of the foliage leaves are visible, and from this time onward their growth, as well as that of the young axis, is rapid and is accompanied by the gradual reflexing of the scales (which bend sharply near their bases) and their ultimate fall from the shoot (Pl. LXIII, fig. 3).

The outer bud scales (*i.e.*, usually the first two) show little or no change in size or color during the expansion of the bud and are early deciduous, leaving narrow, closely-crowded, crescent-shaped scars on the stem. The third and fourth scales are slightly accrescent but offer so much resistance to the more rapidly growing inner scales that they are most frequently irregularly split or cracked (Pl. LXIII, fig. 3).

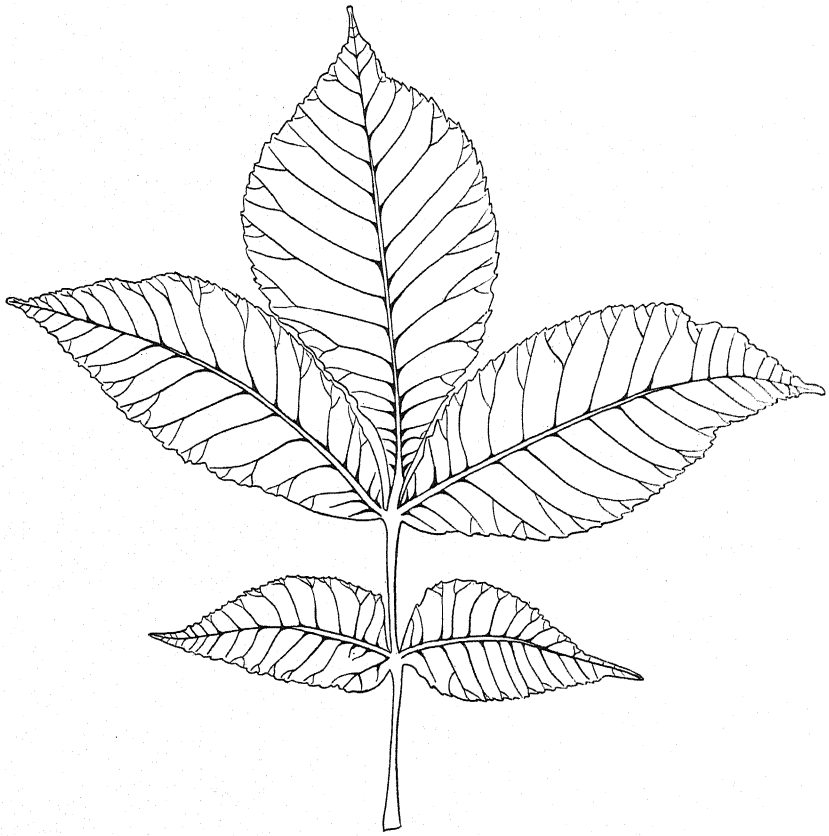
The inner cataphylls, however, not only increase considerably in size but also, as a result of rapid extension in their basal region, acquire a characteristic obovate or spatulate form. One should compare in this connection the form of the sixth and seventh scales in the winter bud (Pl. LXI, figs. 7 and 8) with the adult configuration of these organs (Pl. LXII, figs. 3 and 6). Of general interest is the fact that as the inner cataphylls elongate and reflex, the upper (and hence the broadest) portion of these organs tends to retain the cowl-like character which it exhibited in the winter bud. The color of the inner accrescent scales is quite variable, ranging from a light green tinged with pink (especially on the median and apical regions) to a decided reddish tone, which seems to be particularly characteristic of the cataphylls of expanding buds of coppice shoots. This red or pink color of the adult scales is presumably due to anthocyanin but extensive field observations indicate a decided variability in its appearance, the scales of certain trees possessing large amounts of it, while in others it seems to be almost completely absent.

During the expansion of the terminal bud, the most noticeable evidence of growth in the foliage leaves consists in the rapid elongation of their petioles, which is accompanied by the gradual increase in size and thickness of the pulvinus-like leaf bases. As the foliage leaves emerge from the "tube" formed by the accrescent inner scales, the leaflets are erect, unexpanded, and closely crowded together and no signs of a rachis are evident (Pl. LXIII, fig. 3). When the leaves are completely exposed and the petioles have reached a noticeable length, leaflet expansion occurs, beginning first in the lowest pair of lateral leaflets and proceeding acropetally towards the terminal leaflet. The development of the rachis, which accompanies leaflet expansion, is brought about by intercalary extension between the *lower* pairs of lateral leaflets. The uppermost pair of lateral leaflets and the terminal leaflet are not separated usually but have a common point of attachment to the epipodium.[9]

[9] For an explanation of this term cf. Foster, 1928, p. 157.

Form and Organization of the Adult Foliage Leaf

In contrast to the cataphylls, the adult foliage leaf of the black hickory is a highly complex organ morphologically and consists of three more or less clearly demarcated "elements," viz.: the lamina, the petiole, and the leaf base, the nature of which will be now described (text fig. 2).



Text Fig. 2. Dorsal aspect of an adult foliage leaf, showing the main veins of the leaflets. Notice the relatively short petiole, the jointed rachis, and the pulvinus-like leaf base. (One-half natural size.)

The lamina is of the imparipinnate type and consists of a terminal leaflet and a variable number of pairs of lateral leaflets. Table 1 shows two important facts, viz.: (1) that there is a marked tendency for the number of pairs of lateral leaflets to decrease in the successive leaves of a given shoot and (2) that the total number of leaflets found in a leaf is somewhat correlated with the relative position of that leaf on the shoot. Thus in perhaps the majority of cases, the first leaf of the shoot possesses seven leaflets and successive leaves tend to show a reduction to five or

TABLE 1. *Variation in Number of Leaflets of the Successive Foliage Leaves of the Shoot. Unless Otherwise Indicated the Last Foliage Leaf in Each Tabulation is Followed by the Upper Transitional Forms*

Shoot Number	1st F.L.	2d F.L.	3d F.L.	4th F.L.	5th F.L.	6th F.L.	7th F.L.
1	7	7	7	3			
2	7	7	7	3			
3	7	7	7	7	3		
4	7	7	7	5	5	Female infl.	
5	7	7	7	5	5	3	
6	5	5	5	3			
7	7	5	5				
8	7	5	5	3			
9	7	7	5	5	3		
10	7	5	5	5	Female infl.		
11	7	7	7	5	3	Female infl.	
12	7	5	7	5	3	Female infl.	
13	7	5	5	5			
14	7	7	5	5	5	5	5
15	7	5	5	3			
16	5	5	5				
17	7	7	5	5			
18	5	7	5	5			
19	5	5	5				
20	7	7	5	5			
21	7	7	5	3			
22	7	5	5	5	3	Female infl.	
23	5	7	5	5	3		
24	7	5	5				

three leaflets. If the first leaf develops five leaflets, successive leaves may all show the same organization but not infrequently (table 1, shoot No. 6) the number of leaflets is reduced to three in the last leaf of the shoot. Although a correlation thus appears to exist between the position of a leaf on the shoot and the number of leaflets developed,[10] the "causal factors" responsible for this phenomenon are quite obscure at present.

The form of the leaflets is related to their position in the lamina. The terminal leaflet, which is frequently the largest, is usually obovate-acuminate, bilaterally symmetrical, and shortly petiolate. In contrast, the lateral leaflets are ovate or lanceolate acuminate and are more or less definitely asymmetrical in that their basiscopic halves tend to be the larger.[11] Of interest is the fact that at the point of attachment of the sessile lateral leaflets, the rachis is noticeably swollen, a fact associated with its conspicuously jointed character.

Terminal and lateral leaflets alike possess a prominent dorsal midrib from which are given off, in an alternating pinnate fashion, strong lateral veins which extend through the leaflet blades and, near their edges, fork once or more before sending veins into the prominent marginal teeth (text fig. 2). Text figure 2 fails to illustrate the additional fact that the

[10] A similar reduction in the number of leaflets in successive leaves occurs in *Aesculus Hippocastanum*. Cf. Foster, 1929, p. 449, and table 12, p. 490.

[11] Cf. Diez, 1887, p. 67, for a similar observation on *Carya alba* and *Carya amara*.

main lateral veins are joined together by smaller transverse bundles between which the usual highly complex reticulate system of veins and vein islets appears.

The petiole of the foliage leaf is of the usual dicotyledonous type and presents no features of particular morphological interest. It merges below into the thick pulvinus-like leaf base which is very slightly concave on its ventral surface.

Form and Venation of the Adult Cataphylls

The adult cataphylls of the black hickory stand in striking contrast to the highly segmented foliage leaf; this fact is equally true of the outermost bud scales, which practically reach their full growth by winter, and of the inner scales, which experience an increase in size and an alteration in form during bud expansion. However, when the form and venation of the cataphylls are examined comparatively, a good deal of light is shed upon the nature of the morphological divergence of these organs from the foliage leaves.

The lower, non-accrescent bud scales are ovate-acute and extremely broad-based in form (Pl. LXI, fig. 4). A significant feature of these organs is their more or less prominent dorsal keel which is distinct from the thinner lateral wings. As I shall show later, this bilateral symmetry (or asymmetry), which is externally marked in the outer scales and is particularly well expressed in the venation of the inner cataphylls, is an important consideration in the morphological interpretation of the scale.

In general, the inner cataphylls are relatively narrow based (as compared with the outer scales) and vary from obovate to spatulate or even linear-rhomboid in form. A wide comparative study shows an extraordinary amount of variation in the form of these inner scales; indeed, the illustrations found on Plates LXII, LXIII, and LXIV represent only some of the more common types which occur and incompletely depict the range in configuration which obtains. For example, the fourth scale (Pl. LXII, fig. 2, and Pl. LXIV, fig. 1) as a result of its slight basal extension in the spring, is more or less broadly obovate in form and possesses a rather distinct emarginate apex; in some instances, the emarginate character is so strongly developed that the upper portion of the scale is somewhat bilobate. Of interest is the fact that in some scales of this type, a tiny pointlet is present at the base of the emargination, whilst in others it is absent. The fifth scale (Pl. LXIV, fig. 2) while larger and with a somewhat narrower base, is usually similar to the fourth scale in general form. The sixth scale, however, is definitely variable in form. In some buds (Pl. LXII, fig. 3) this organ possesses a relatively slender stalk-like portion which gradually widens out above into an acute, irregularly lobed, blade-like portion. Not infrequently, however, the sixth scale is broadly spatulate in form with a more or less definitely truncated apex (Pl. LXIV, fig. 3); as in the emarginate

type of scale, a definite apical point may be present or absent in connection with this type of scale.

The seventh or last cataphyll of the bud without doubt exhibits the greatest polymorphism of any of the scales. Perhaps most frequently this organ is narrowly or broadly spatulate and clearly asymmetrical (Pl. LXII, figs. 5*a* and 6; Pl. LXIII, figs. 4*a* and 5*a*; and Pl. LXIV, figs. 5, 6, and 7). With such a configuration, the seventh scale frequently bears a striking resemblance *in form* to a small, simple, obscurely petiolate foliage leaf. In other instances, however, the last cataphyll of the bud is linear-obovate or strap shaped (Pl. LXII, fig. 4*a*; Pl. LXIV, fig. 4); of interest is the fact that cataphylls of this type, when reflexed, are often coiled and contorted in various ways. A comparatively infrequent form of the seventh scale is illustrated by figures 8 and 9 of Plate LXIV; these organs are linear-rhomboid in form and usually larger than any of the other types.

A not unusual feature of the seventh cataphyll is the presence of a more or less clearly demarcated "terminal lobe." The relation of this to the morphology of the cataphyll will be discussed later; for the present, I shall confine myself to a description of its form and general characteristics. When viewed dorsally in a spatulate type of scale which has been spread flat for study, the terminal lobe is somewhat ovate or deltoid-acute in form and quite distinct from the broad, irregularly crenate or toothed shoulder-like wings of the cataphyll (Pl. LXII, figs. 5*a*, 6; Pl. LXIII, figs. 4*a* and 5*a*). In the linear type of scale, the terminal lobe is less clearly demarcated and is only slightly wider than the rest of the cataphyll (Pl. LXIV, fig. 4). The terminal lobe of the linear-rhomboid type of cataphyll (Pl. LXIV, figs. 8 and 9) is fairly distinct due to a more or less definite constriction between it and the upper, broadly-winged portion of the scale. When viewed *ventrally*, one finds that the margins of the terminal lobe are more or less prominently involute (Pl. LXII, figs. 4*b* and 5*b*); this condition appears to be retained as long as the cataphyll remains attached to the shoot. An extremely interesting characteristic of the terminal lobe is its dark green color which contrasts very noticeably with the much lighter color of the remainder of the scale. This feature is particularly obvious in scales possessing anthocyanin, in which case the terminal lobe and dorsally prominent median central region of the scale are dark green whilst the cataphyll wings are a delicate pink in color.

During the course of this investigation, an effort was made to discover possible correlations which might throw some light upon the meaning of the extraordinary polymorphism of the inner bud scales but negative results were obtained. That is, the form of the cataphyll appears to be independent of its size or position in the shoot system of the bud from which it develops; likewise, the configuration of the scale seems to stand in no direct relationship to the vigor of the shoot upon which it develops. Two examples will serve to support these conclusions: (1) The obovate-emarginate form, as

previously stated, is usually found in the fourth and fifth scales (Pl. LXIV, figs. 1 and 2), the upper scales (*i.e.*, the sixth and seventh) showing a decided tendency to the development of a truncate or acute apex or of a definite terminal lobe. However, in many normal expanding buds I have observed all the *uppermost* cataphylls of the obovate-emarginate type. (2) In *nearly every* opening terminal bud of a small roadside tree (apparently normal and healthy) the seventh scale possessed a dark green conspicuous terminal lobe. In other trees, similar in size and vigor, scales with definite terminal lobes are the exception rather than the rule.

A correlation of a different order, however, does exist between the *form* and the *venation* of the cataphylls and a wide study of this relationship has brought forward a number of important facts which bear directly upon the question of the organization of the cataphyll and the nature of its terminal lobe.

Before discussing the venation of the cataphylls, it will be necessary to point out that both scale and foliage leaf in the black hickory possess a trilacunar node.[12] Reference to text figure 1 shows the departure from the stele of the vascular supply of the third foliage leaf of the bud. It will be noted that the median trace is tripartite, a characteristic also of the smaller median bud-scale traces. In the mature foliage leaf, the leaf traces, together with additional vascular tissue, form a stelar system which extends through the petiole and rachis and from which separate bundles are given off to each lateral leaflet. In contrast, the traces to each scale tend to break up rather soon in the bases of these organs, each of which, in transverse section, shows a crescentic series of vascular bundles located near its ventral surface (text fig. 1).

In general terms, the venation of the cataphylls is of the palmate-dichotomous type, with a more or less prominent series of median veins (or a midrib) and numerous repeatedly forking diverging lateral veins which innervate the wings of the cataphyll. Since both the quantitative and qualitative character of the venation is related to the form of the cataphyll and, in part, to its position in the shoot, the facts must be presented comparatively.

The fourth, fifth, and sixth scales are traversed by a complex series of more or less palmately-diverging, dichotomizing veins which not infrequently are connected at certain points by oblique anastomoses [13] (Pl. LXIV, figs. 1–3). Upon close examination, it will be noticed that the *outer* lateral veins supplying the wings of each scale are distinct and can be traced to its base (where they join together as the lateral scale traces) whilst the *inner* lateral veins arise as branches from the two lateral cords of a trio of

[12] This statement is probably true for the majority of cases although variations in the nodal anatomy of the scale possibly occur. In general, the vascular supply of bud scale and foliage leaf tends to be identical in the Angiosperms (cf. Foster, 1927).

[13] The amount of anastomoses appears to be related to the size and vigor of the scale, being most numerous in large cataphylls.

median bundles extending from base to apex.[14] In figures 1 and 2 of Plate LXIV, it is evident that the mid-vein of this median trio of bundles runs from base to apex without branching; in the sixth scale, however (Pl. LXIV, fig. 3), the mid-vein sends off two lateral veins in its course through the scale. Thus, in spite of the seemingly complex nature of the vein system in these scales, there is a definite indication of midrib organization (further shown by the darker green color of the median central region of the scale) and consequently the appearance of bilateral symmetry.[15]

A number of important modifications in the general scheme of venation appear in the innermost cataphylls and seem to be correlated in the first place with the relatively narrow bases and the various configurations of these organs. One of the most prominent of these modifications is the differentiation of a more or less distinct, pinnately branching midrib (Pl. LXIV, figs. 4, 5, 6 and 7). Associated with the development of a definite midrib, one finds a noticeable reduction in the number of separate lateral veins (which now are obviously derived from the lateral scale traces) and a marked tendency for the bulk of the vein system to be derived fundamentally from the midrib itself.

Figure 5 of Plate LXIV illustrates in a striking fashion some of the changes in venation which appear, naturally in varying degrees, in the innermost scales. This figure shows that a prominent midrib (shown in solid black), extending nearly the complete length of the narrow portion of the scale, sends off alternately four main dichotomizing branches into the lateral wings of this organ. Just below the broadened portion of the scale, the midrib divides into three veins, two of which extend into the left wing and fork near its margins while the third vein remains unbranched and extends into the apical pointlet. This "trifurcation" of the midrib is characteristic of the innermost scales (Pl. LXIV, figs. 5, 6, and 7) and represents an important line of evidence in the morphological interpretation of the terminal lobe, as will be shown later. Figure 5, Plate LXIV, likewise illustrates the decreased number of separate lateral veins found in the base of the seventh scale. At the right of the midrib, two lateral veins, which clearly represent branches of a lateral scale trace, are evident; it will be seen that the inner of these two veins forks and that between it and the outer unbranched lateral vein there occurs an isolated vascular strand. A similar strand likewise occurs close to the lower left-hand margin of this scale.[16]

Figures 6 and 7 of Plate LXIV illustrate further modifications in venation

[14] The terms used in describing the course, branching, etc., of the veins are descriptive in nature and do not imply the actual mode of origin of the vascular system of the scale, which remains to be investigated.

[15] A wide study indicates that the trio of median bundles represents the tripartite median scale trace.

[16] Such isolated strands suggest a basipetal maturation of the smaller veins of the scale. Bugnon (1928, p. 43) figures a similar condition in the hypsophylls of *Ribes sanguineum*.

associated with the narrow-based spatulate type of scale. In figure 7 one can see the highly simplified character of the primary lateral veins and the pinnate branching of the prominent midrib which trifurcates a short distance below the apex of the scale, forming two forked lateral veins and an unbranched median which runs into the apical pointlet. The venation of the scale shown in figure 6, Plate LXIV, suggests a simple, palmately-veined, foliage leaf (except that the finer reticulate vein system is lacking) and represents an extreme expression of midrib development in the cataphylls.

In cataphylls possessing a definite terminal lobe, the vein system is of particular interest. Plate LXIII, figure 4*b*, depicts the venation of the apical region of the scale shown in figure 4*a*. It will be seen that the deltoid-acute terminal lobe is largely innervated by the three main veins which have resulted from the trifurcation of the midrib. This situation is fundamentally identical with the condition in other cataphylls where a definite terminal lobe is absent (cf. fig. 4*b*, Pl. LXIII, with fig. 7, Pl. LXIV). In cases where the terminal lobe is very clearly demarcated (Pl. LXIII, figs. 5*a* and 5*b*), its venation is slightly more complex but as before, the three main veins of this structure have resulted from the trifurcation of the midrib.

However, if one now examines the venation of the linear-obovate or linear-rhomboid type of cataphyll, certain additional facts appear which give meaning to this characteristic trifurcation of the midrib and likewise shed light upon the nature of the terminal lobe. One of the most significant features of the terminal lobe in these organs is the appearance of a weakly developed but definite reticulate venation.[17] Figure 8, Plate LXIV, for example, illustrates a typical condition. The lower portion of this organ is innervated by veins derived from the primary lateral bundles as well as from branches of the prominent midrib; in this respect, the vein system is similar to that shown in figures 5, 6, and 7 of Plate LXIV. Some distance below the narrowed terminal portion of this organ, the midrib trifurcates and forms three prominent veins which behave as follows: the median vein remains unbranched and extends into the acute tip of the terminal lobe while the two lateral veins each form a few branches from which are derived, in the *lateral portions* of the terminal lobe, a weak network of small, anastomosing veinlets. A fundamentally similar condition is illustrated in figure 9 of Plate LXIV, the main differences being the more complex behavior of the three veins derived from the midrib and the greater development of the network of veinlets in the terminal lobe. Figure 4, Plate LXIV, illustrates a type of venation associated with a narrowly-linear seventh scale in which the lateral wings are greatly reduced in extent and the median, dorsally ribbed region is correspondingly well developed. It will be seen from this

[17] Additional observations made during the spring of 1931 show that reticulate venation may appear in the terminal lobe of the spatulate type of scale also.

figure that the two primary lateral veins (which represent the lateral scale traces) are unbranched and that the bulk of the vein system is derived from branches of the midrib. The mode of innervation of the terminal lobe is somewhat more complex than in the types previously considered but is based upon the same general principle, *i.e.*, the trifurcation of the midrib. A considerable distance below the apex of this organ, a pair of prominent lateral veins diverge at either side from the midrib and give rise to a network of veinlets in the lower lateral portions of the terminal lobe. Just above the point of separation of these two lateral veins, the midrib trifurcates; the two lateral bundles resulting contribute further, by subdivision, to the network of veinlets in the terminal lobe whilst the median vein, after forming a weak branch, extends into the apical pointlet.

The development of reticulate venation in the so-called terminal lobe of certain cataphylls suggests that this structure may represent a poorly delimited *laminar region*. Further support is lent to this suggestion by the occasional development, in precociously expanding buds, of such an organ as is shown in figure 10, Plate LXIV. This cataphyll consists of a slender, asymmetrical, curved, petiolar portion terminating in a small, obscurely crenate lamina. The venation of this organ offers definite points for comparison with the situation in scales of the type illustrated by figures 4, 8, and 9 of Plate LXIV. One finds that the midrib, as in the inner scales, trifurcates near the base of the lamina; the resulting veins form a very weak network which is further increased by the formation of additional veinlets by the main primary lateral vein at the left of the midrib.[18] Further discussion of the relation of venation to the morphology of the scale will occur in the following section of this paper.

The Morphology of the Cataphyll

It seems extraordinary that the conspicuous bud scales, not only of the black hickory but also of other more widely distributed species,[19] should have attracted so little morphological investigation in the past. Sargent (1922, p. 184 and p. 187), for example, refers to the general form, size, color, and persistence of the accrescent inner cataphylls in such species as *C. ovata* K. Koch and *C. laciniosa* Schn. but expresses no opinion as to their morphology. Indeed, O. F. Cook appears to be one of the few investigators who has offered any suggestions as to bud scale organization in *Carya*. In an early paper, Cook (1916, p. 542) makes the following statement: "The large membranous bud scales that precede the leaves on new shoots of *Hicoria* also represent sheaths and sometimes appear in modified form as stipular wings of the petiole, on abnormal leaves of shoots developed

[18] It is instructive to compare the venation of the lower portion of this organ with that of the scales shown in figures 6 and 7 of Plate LXIV.

[19] For example, *Carya ovata* K. Koch, *Carya alba* K. Koch, *Carya laciniosa* Schn. and *Carya glabra* var. *megacarpa* Sarg.

late in the season." More recently, Cook (1923, p. 84) has advanced the theory, based largely on a study of aberrant leaf forms, that the compound leaves of *Juglans* and *Carya* "appear to have been developed, not by subdivision of the original simple leaf but by reduplication of the foot element, with its stipular elements, to form the several joints of the rachis and the corresponding pairs of pinnae." The expression "foot element" is used by Cook to designate the "primitive sheathing leaf base" which he supposes is conspicuously developed in the bud scales of walnuts and hickories.[20]

In support of his theory Cook (1923, fig. 9) figures a number of interesting intermediate leaf forms in *Carya*,[21] which vary from organs consisting of a winged basal region (the wings forming two stipular-like lobes above) terminating in a small blade, to forms in which a narrowly-winged petiolar region is surmounted by a more highly segmented lamina.

It is of considerable interest at this point to note the correspondence between Cook's idea of the morphology of hickory cataphylls and the general theory of leaf evolution advanced by Domin (1931). As I have already shown in detail (Foster, 1931, pp. 244–246), Domin (1911, p. 18) attempts to demonstrate that in many exstipulate dicotyledonous families, among which he includes the Juglandaceae, the supposedly primitive "lower member" or sheath (*i.e.*, Cook's "foot") appears only in certain foliar organs, especially cataphylls, and is "reduced" to a petiole in the foliage leaves. Sufficient reasons exist, in my opinion, for considerable doubt as to the correctness of this phylogenetic viewpoint of cataphylls which forces us to make certain *a priori* and unproved assumptions regarding the nature of the primitive angiosperm leaf (cf. Foster, 1928, pp. 152–156).

When one attempts now to apply Cook's theory to the situation in *Carya Buckleyi* var. *arkansana* difficulties, both theoretical and practical, appear. Intermediate leaf forms, of the type described by Cook, do occur in this species on vigorous stump-shoots or in precociously expanding buds. Text figure 3 illustrates one of these remarkable organs and shows that the thin lateral petiolar wings are joined with the dorsal surface of the midribs of the lowermost pair of leaflets.[22] In my judgment, however, it would be an error to cite such organs (the developmental relations of which are quite unknown) as "proof" that the *normal* cataphylls of the black hickory correspond morphologically to sheaths. Foliar organs of the type shown in text figure 3 merely illustrate, in my opinion, that under somewhat disturbed developmental conditions, *lamina formation*, which is the marked feature of the foliage leaf, and *wing formation*, which is characteristic of the cataphyll, have simultaneously appeared in the same organ. Such foliar

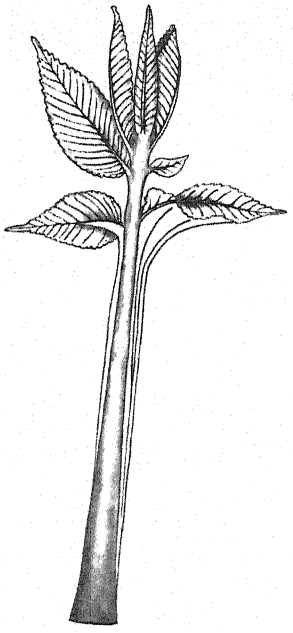
[20] For further information on the meaning of the term "foot" cf. Cook (1916, pp. 544–545).

[21] Unfortunately, Cook fails to list definitely the species of hickory investigated although one reference (*op. cit.*, p. 81) indicates that *Carya pecan* Asch. & Gr. was studied.

[22] Cook (1923, p. 81) has noted this condition in the pecan.

structures, however, fail to give a convincing proof of the relative antiquity of either type of configuration (cf. also Foster, 1931, p. 246).

Aside from any theoretical objections to Cook's interpretation the polymorphic form and the palmate dichotomous venation of the adult cataphylls of the black hickory indicate clearly that these organs are not morphologically homologous with "sheaths." On the contrary, I have reached the conclusion that, in this species, the cataphylls represent *unit*



TEXT FIG. 3. Dorsal aspect of the third foliar organ of a precociously expanding terminal bud of a coppice shoot, April 23, 1930. Notice particularly that the thin, unequal petiolar wings are adnate to the midribs of the lowest pair of lateral leaflets. The second pair of leaflets are unequal in size while the uppermost pair and the terminal leaflet have not yet expanded. Total length of this organ, 31 mm.; length of winged portion, 17 mm.: basal width, 3 mm. (× 2.5).

foliar organs with an extremely simple morphological organization which usually precludes any *direct* homology with the elements of the adult foliage leaf. The salient features of the evidence supporting this conclusion are as follows.

In view of the progressive and often gradual change in form and venation of the successive cataphylls of a single bud (Pl. LXIII, fig. 3), the broad-based, vaginate form of the outer bud scales is clearly *analogous* to that of a "sheath" rather than *homologous*. Aside from the fact that the foliage leaf exhibits no sheath-like "element" with which one of these

lower scales (Pl. LXIV, fig. 1) might be compared, the venation of these organs clearly suggests bilateral symmetry with a noticeable tendency for a distinction of *lateral wings* and an *axial region*.[23] If one now regards the obovate or spatulate form of the inner scales, with their more or less prominent pinnately-branching midribs, their greatly reduced primary veins and their relatively narrow bases, any analogy of such organs to a "sheath," using this term in its generally accepted morphological sense, clearly vanishes. Such an organ, for example, as is shown in Plate LXIV, figure 6, suggests a simple, palmately veined, foliage leaf. The architecture of the cataphylls is particularly well illustrated in such inner bud scales as are shown in Plate LXIV, figs. 5, 6, and 7, where the fundamental organization is that of a foliar organ possessing a more or less clearly demarcated axial region [24] flanked by lateral, often asymmetrical, wings.

The question now arises as to the morphological nature of the cataphyll wings. Tyler (1897, p. 39 and fig. 42) interprets the wings of the transitional leaves of *Carya alba* K. Koch and *Carya microcarpa* Darling as "adnate stipules" and Cook (1923, p. 81) likewise ascribes a stipular character to the "wings of the leaf base" of certain intermediate leaves in *Carya*. In view of the exstipulate character of the foliage leaves of the Juglandaceae as a whole, however, it appears very doubtful to me that the wings of the cataphylls and transitional forms in the black hickory represent stipules. In my opinion, the cataphyll wings simply represent the lateral portions of a morphologically and anatomically simple leaf. The petiolar wings of the intermediate leaf forms (text fig. 3) are clearly of the same nature as the thin lateral portions of the cataphylls but I regard them as structures resulting from the persistence of the marginal growth characteristic of the scale, rather than as indicators of a "primitive" type of leaf (cf. Cook, 1923, p. 88).

In perhaps the majority of buds, the transition from the last cataphyll to the first foliage leaf is abrupt (cf. Pl. LXIII, fig. 3) and hence the wide morphological divergence between scale and leaf is extremely obvious. Certain cataphylls (Pl. LXIV, figs. 4, 8, and 9), however, exhibit an imperfectly demarcated and poorly differentiated laminar region, in which a small amount of reticulate venation occurs. The mode of innervation of this laminar region as compared with the apical venation of the more typical cataphylls (Pl. LXIV, figs. 5, 6, and 7) and the continuity of its usually entire margins with the wings of the scale, are facts clearly indicating the purely arbitrary demarcation of morphological elements which can be drawn even in these organs. One may conclude, therefore, that while "polarity," as expressed in the differentiation of a definite lamina, is usually absent in the cataphylls, it may be operative in an imperfect degree, leading to the type of scale shown in Plate LXIV, figures 4, 8, and 9.

[23] The axial region of the cataphyll might be considered to represent the "phyllopodium" or leaf axis (cf. Foster, 1928, p. 157).

[24] In the upper cataphylls, the axial region is indicated by a more or less prominent median dorsal rib.

Further consideration of the problem of the form and organization of the cataphylls and foliage leaves in the black hickory must be deferred until the following paper of this series where it will be shown that the facts revealed by ontogeny support the morphological interpretation of the scale which has just been advanced and in addition throw considerable light upon the form divergence between the cataphyll and the foliage leaf.

Summary

1. The shoot system of the black hickory is of the monopodial-racemose type and shows a clear distinction of rapidly growing "long shoots" and slowly growing "spur shoots." On both types of shoots, a periodic alternation of cataphylls and foliage leaves obtains which is broken only by the appearance of the terminal female inflorescence.

2. Vegetative terminal buds of the long shoots consist of a short axis bearing 9–11 cataphylls, 5–11 foliage leaves, and usually the primordia of the two outer scales of its next season's bud. The vegetative terminal buds of the dwarf shoots develop a smaller and more constant number of cataphylls (9) and foliage leaves (3–4).

3. In "mixed" terminal buds of both "long" and "spur" shoots, well-developed staminate catkins are present during the winter in the axils of the upper cataphylls, and the axis of the bud terminates in either an embryonic female inflorescence or, as in the vegetative buds, in the primordium of the next season's terminal bud.

4. The two outer cataphylls of the terminal bud are designated as the "upper transitional forms" since they consist of a dorsally keeled, winged basal portion surmounted by a more or less distinct rudimentary laminar primordium. In contrast the bud scales proper are vaginate in form with no trace of laminar differentiation.

5. The foliage leaves of the winter bud consist of a well-developed lamina of 5–7 convolute-involute leaflets, a short petiolar region, and a broadened leaf base.

6. During the expansion of the terminal bud, the inner accrescent scales form a tube-like structure enclosing the cluster of enlarging foliage leaves. Eventually, the cataphylls reflex and fall from the new shoot. The growth of the foliage leaves is accompanied by the rapid elongation of the petiole followed by the acropetal expansion of the leaflets and the development of the rachis.

7. The adult foliage leaf is highly complex morphologically, consisting of an imparipinnate lamina of 3–7 leaflets, a petiole, and a pulvinus-like leaf base. Successive foliage leaves of the shoot frequently show a progressive reduction in the number of pairs of lateral leaflets, from 7 to 5 or 3.

8. The adult lower bud scales are ovate-acute, or ovate-obovate emarginate in form. The upper scales vary greatly in form, common types being obovate, spatulate, linear-rhomboid, and linear.

9. Certain cataphylls develop a "terminal lobe" which is shown to represent a poorly differentiated laminar region.

10. The node of both cataphyll and foliage leaf is trilacunar, with the median trace tripartite. The vascular tissue is arranged in a "stele" in the petiole of the foliage leaf whilst the venation of the cataphylls is of the palmate-dichotomous type with a pinnately-branching midrib appearing in the spatulate, linear-rhomboid, and linear types of scales.

11. Cook's interpretation of hickory cataphylls as "primitive sheaths" is rejected for the black hickory and the conclusion reached that in this species the cataphylls represent unit foliar organs with an extremely simple morphological organization which usually precludes any direct homology with the elements of the adult foliage leaf.

12. The fundamental architecture of the cataphyll is that of an organ possessing a more or less clearly demarcated axial region flanked by two lateral, often asymmetrical, wings. The cataphyll wings are regarded as the lateral portions of a morphologically and anatomically simple foliar organ, and not as stipules.

I am deeply grateful to my wife, Helen Vincent Foster, for her painstaking and skillful preparation of the illustrations used in this paper and for her assistance in proof-reading. My thanks are due also to Doctor Paul B. Sears for his many useful suggestions offered during the course of the investigation.

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EXPLANATION OF PLATES

PLATE LXI

FIGS. 1–3. Variations in the form and organization of the upper transitional forms April 9, 1930. FIG. 1, ventral aspect of the outer of two upper transitional forms showing a pair of rudimentary lateral leaflets and slight blade development on the ventral surface of the acuminate terminal leaflet (× 20). FIG. 2, ventral aspect of the outer of two upper transitional forms, showing the absence of lateral leaflet sand the continuity of wing development throughout this organ (× 28). FIG. 3, ventral-lateral aspect of the inner of two upper transitional forms, showing the less prominent apical point and the more obvious vaginate form of this organ (× 42).

FIGS. 4–8. Successive cataphylls of the terminal winter bud illustrating the gradual change in form and size of the organs in such a series. Figure 4 on April 12, 1930; remaining figures (including figure 9) on December 12, 1929. For purposes of clarity, the colleters and the majority of the hairs are omitted in the figures. FIG. 4, dorsal aspect of the first scale, showing its characteristic, ovate-acute, broad-based form and its prominent dorsal keel (× 5). FIG. 5, lateral-ventral aspect of the second bud scale, showing its massive wings and inconspicuous apical pointlet (× 7.5). FIG. 6, lateral-ventral aspect of the third scale, often the broadest and largest scale of the winter bud(× 8.5). FIG. 7, ventral-lateral aspect of the sixth scale, showing the convolute wings and the slight basal asymmetrical constriction of this organ, which is obovate-acute in form (× 10). FIG. 8, ventral-lateral aspect of the seventh and last scale of the bud. Note the comparatively narrow base of this obovate-acute organ. During bud expansion, vigorous intercalary extension occurs in the definitely constricted basal region of this cataphyll (× 12.5).

FIG. 9. Dorsal and lateral aspects, respectively, of the first foliage leaf and the last bud scale, showing the divergence in form and organization between these organs. The foliage leaf consists of seven well-developed leaflets, a short petiole and a broadened leaf base; the cataphyll is simple in form and organization. Note that the scale is only partially imbricated over the foliage leaf (× 12.5).

PLATE LXII

FIGS. 1–6. Variations in the form of the adult accrescent scales of terminal buds expanding on April 12, 1930. All figures, with the exception of figures 4*b* and 5*b*, represent the dorsal outline of the flattened scales. FIG. 1, the general form of the slightly accrescent third scale (× 6). Compare with figure 6, Pl. LXI. FIG. 2, the obovate-emarginate form of the fourth scale (× 2.5). FIG. 3, the broadly obovate form of the sixth scale.

Note the irregularly crenate margins and somewhat lobate character of the upper portion of this organ (× 2.5). FIG. 4*a*, the linear, asymmetrical form of a seventh scale, with a poorly demarcated terminal lobe (× 3). FIG. 4*b*, ventral aspect of the terminal lobe of figure 4*a*, showing the slightly curled margins (× 2.5). FIG. 5*a*, the characteristic spatulate form of the seventh scale. Note the prominent asymmetry of this organ, and its clearly demarcated terminal lobe (× 2.5). FIG. 5*b*, ventral aspect of the terminal lobe of figure 4*a*, showing the inrolled margins (× 3). FIG. 6, the spatulate asymmetrical form of a seventh scale, with irregularly crenate or toothed upper margins and a broad, prominent terminal lobe (× 2.5).

PLATE LXIII

FIG. 1. A long shoot with its terminal and axillary buds, November 8, 1930 (× 1.75). At the base of the shoot, the ring-like bud-scale scars are evident; directly above the uppermost scale-scar is the oval scar of a staminate catkin. Notice the tiny serial bud at the third foliage-leaf node (from below) on the stem. The terminal bud is ovoid-acute and the narrow scar at its base marks the former position of an upper transitional form.

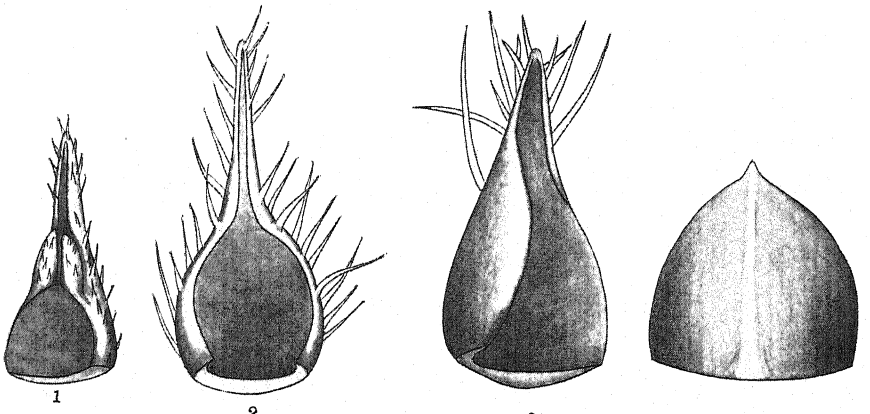
FIG. 2. The upper portion of a spur shoot, March, 1931 (× 3). The characteristic alternation of bud-scale and foliage-leaf scars clearly demarcates the growth made during 1929 and 1930. Note the smaller number of foliage leaf scars and the shorter internodes as compared with the long shoot (figure 1). The terminal bud exhibits three exposed cataphylls of which the one at the right is a dorsally keeled, acuminate upper transitional form. The narrow scar at the base of the bud has been caused by the outer deciduous upper transitional form.

FIG. 3. The opening terminal bud of a spur shoot, April, 1930, showing the emerging cluster of foliage leaves partially enclosed within the "tube" formed by the accrescent inner scales (× 1.5). The distribution of colleters (represented by small circles or ovals) and pubescence on the cataphylls is represented schematically. Notice the irregularly split apices of certain of the lower bud scales. The upper transitional forms and the first bud scale (the scar left by the latter is shown at the base of the bud to the right) have fallen.

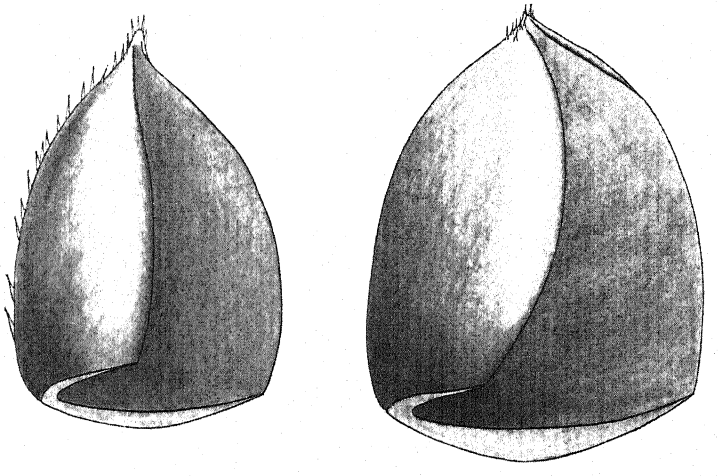
FIGS. 4 AND 5. The dorsal form and ventral apical venation of the seventh scales of two terminal buds expanding April 12, 1930. FIG. 4*a*, a spatulate, asymmetrical type, with a prominent tooth at the right margin and a deltoid terminal lobe (× 3). FIG. 4*b*, outline of the upper ventral aspect of figure 4*a*, showing the apical venation and mode of innervation of the terminal lobe (× 3). FIG. 5*a*, a broadly spatulate type, showing a relatively narrow base, irregularly crenate and toothed upper margins, and a prominent ovate-acute terminal lobe (× 4). FIG. 5*b*, outline of the upper ventral aspect of figure 5*a*, showing the apical venation and the mode of innervation of the terminal lobe (× 5.5).

PLATE LXIV

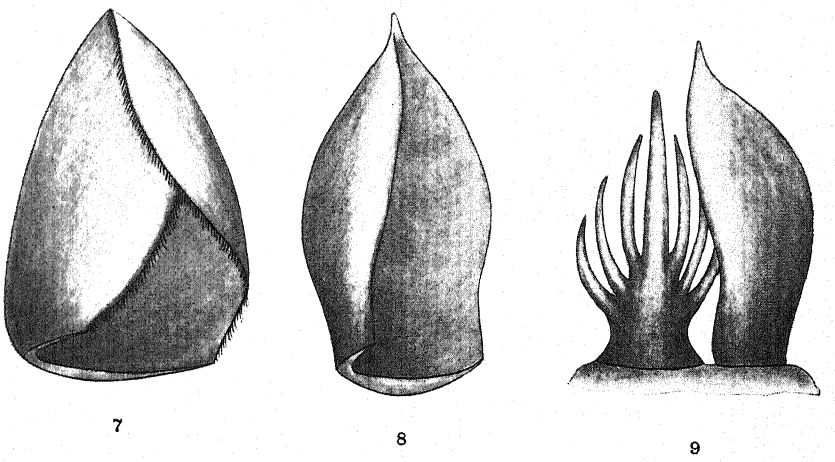
FIGS. 1–10. The form and complete venation of various adult cataphylls. In figures 4–9, the heavy black median line corresponds to the prominent midrib of these organs which is composed of a "complex" of bundles. FIG. 1, the obovate-emarginate form and the venation of the fourth scale. Note the unbranched character of the median of the trio of prominent veins extending through the center of this organ (× 2.5). FIG. 2, the obovate-emarginate form and the venation of the fifth scale. Note that the inconspicuous apical pointlet is situated between two prominent shoulder-like lobes. The median vein of the trio extending through the mid-region of this organ is unbranched (× 2.5). FIG. 3, the spatulate-truncate form and the venation of the sixth scale (× 2.2). FIG 4, the linear-spatulate form and the venation of a seventh scale. Note the reticulate venation of the terminal lobe of this cataphyll (× 2.5). FIG. 5, the spatulate-truncate form and venation of a seventh scale. The dichotomizing veins in the upper portion of this organ are all fundamentally derived from primary branches of the midrib (× 2.6). FIG. 6, the spatulate-acute form and the venation of a seventh scale, with rather simple pinnate



1 2 3 4

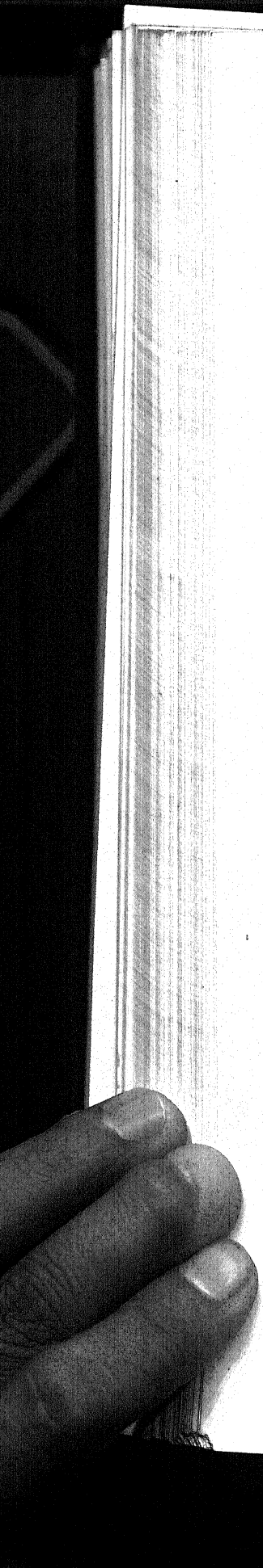


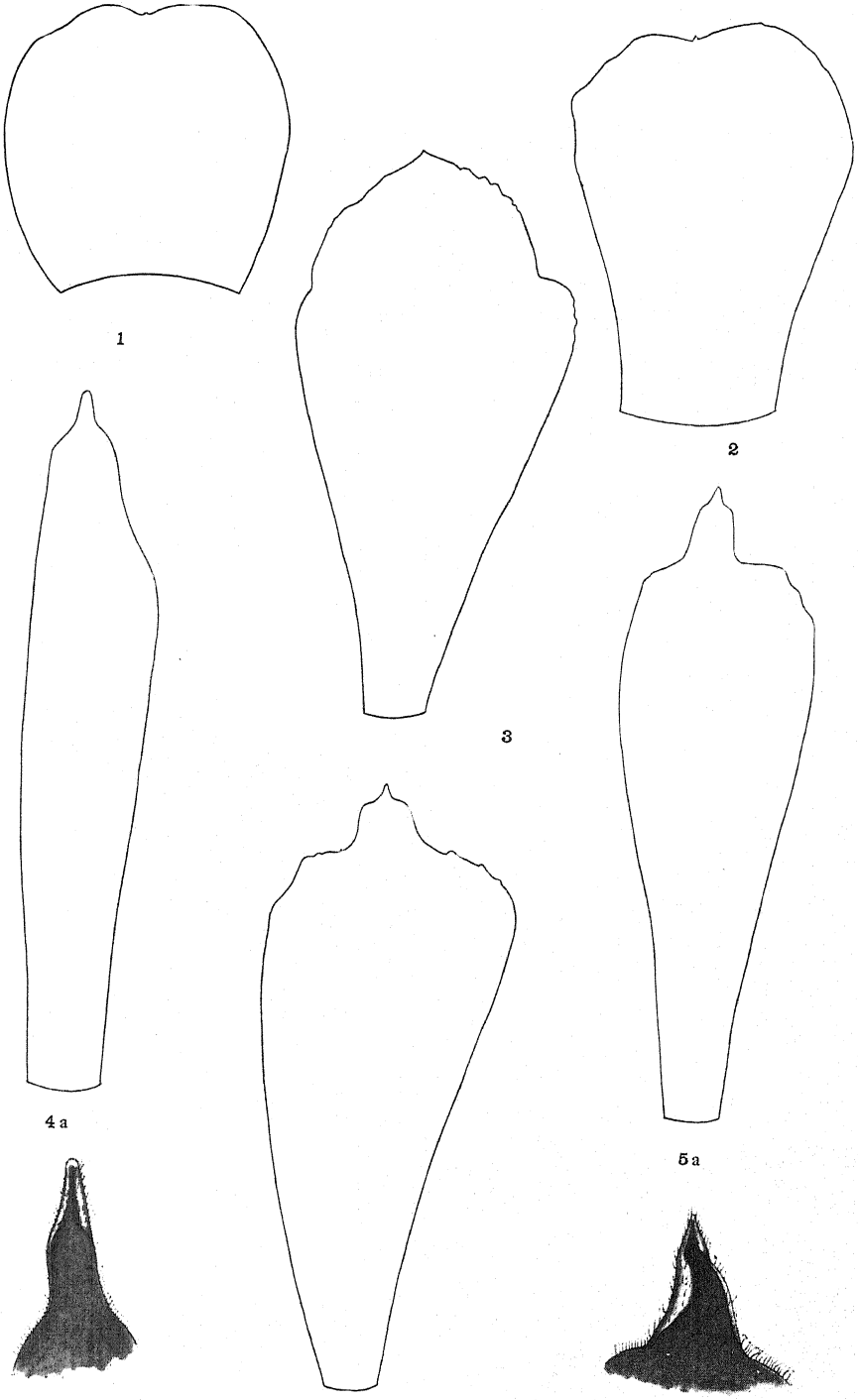
5 6



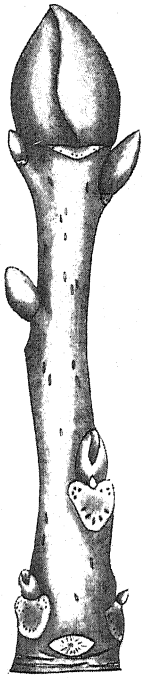
7 8 9

FOSTER: LEAF OF CARYA

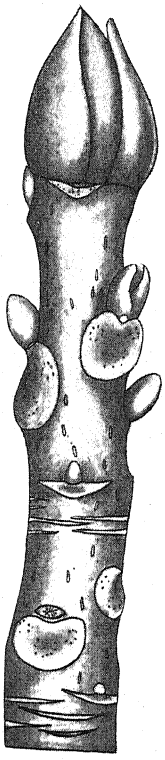




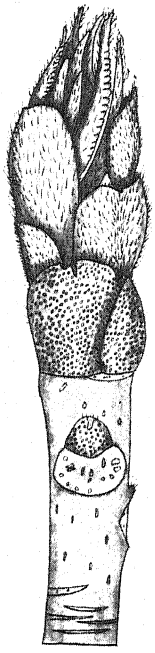
FOSTER: LEAF OF CARYA



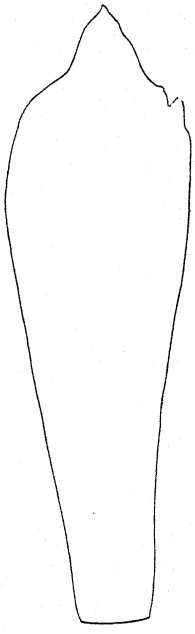
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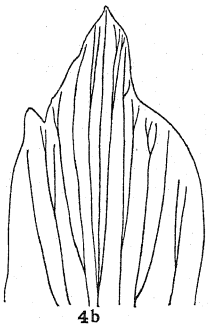
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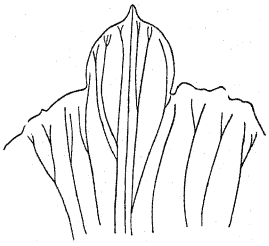
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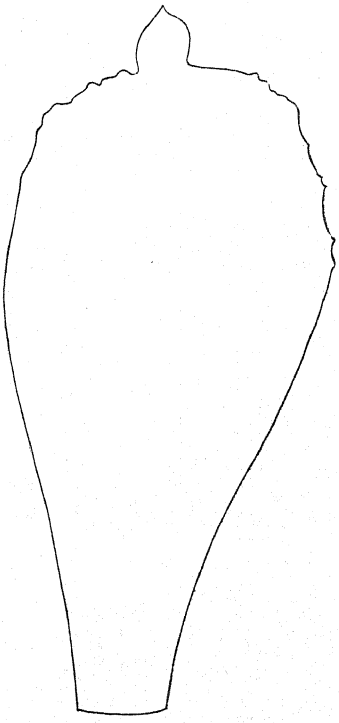
4a



4b

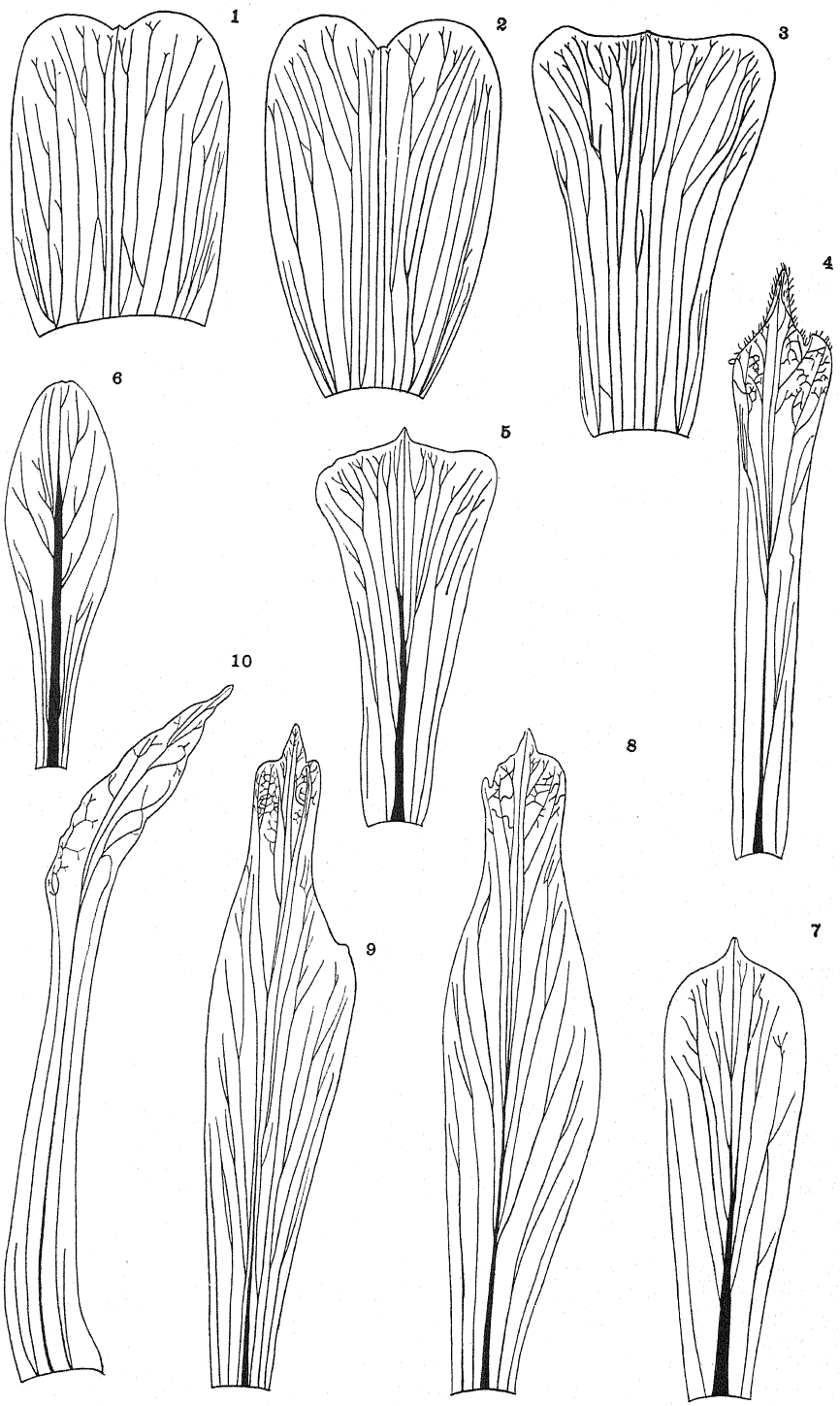


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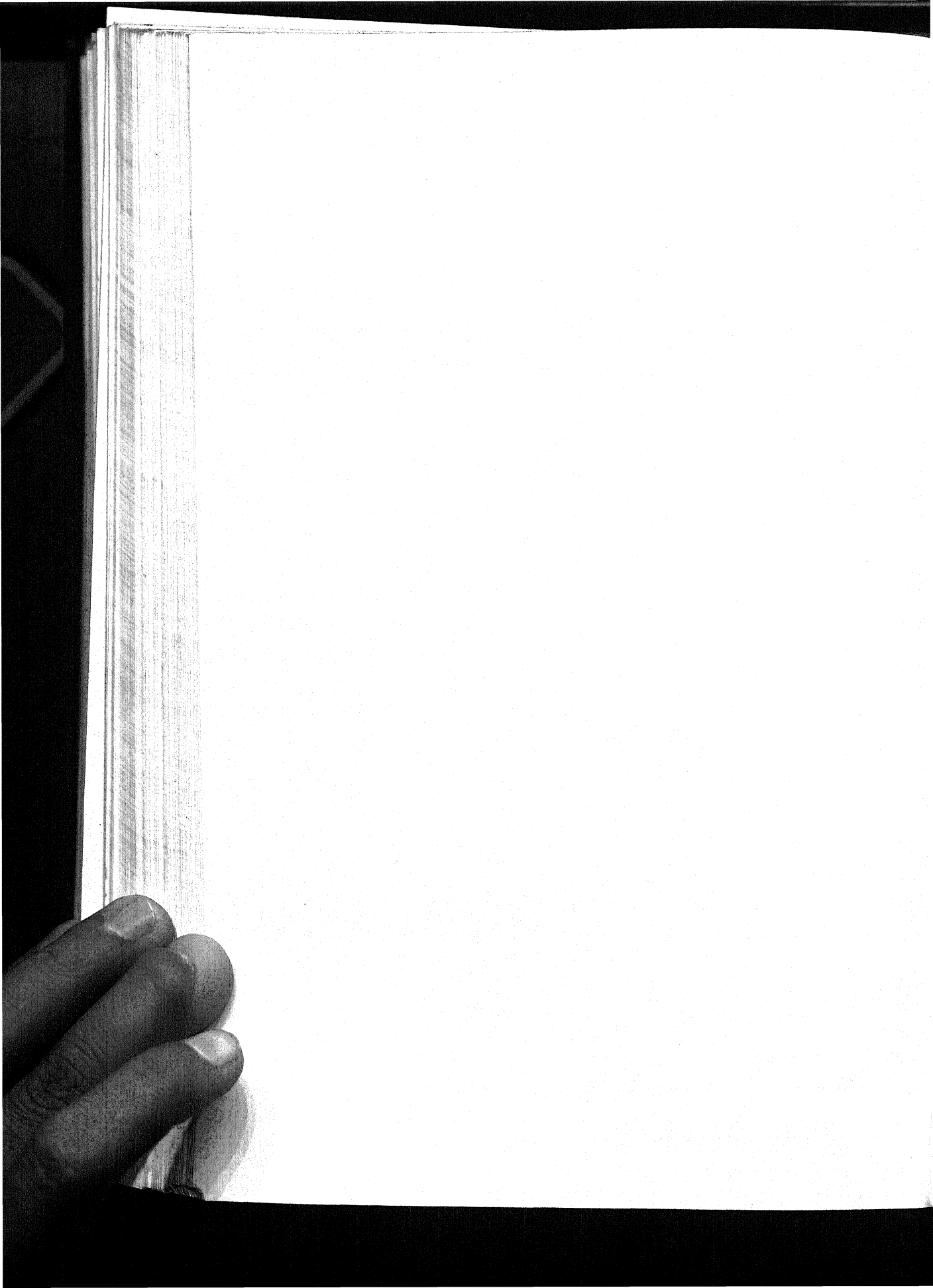


5a

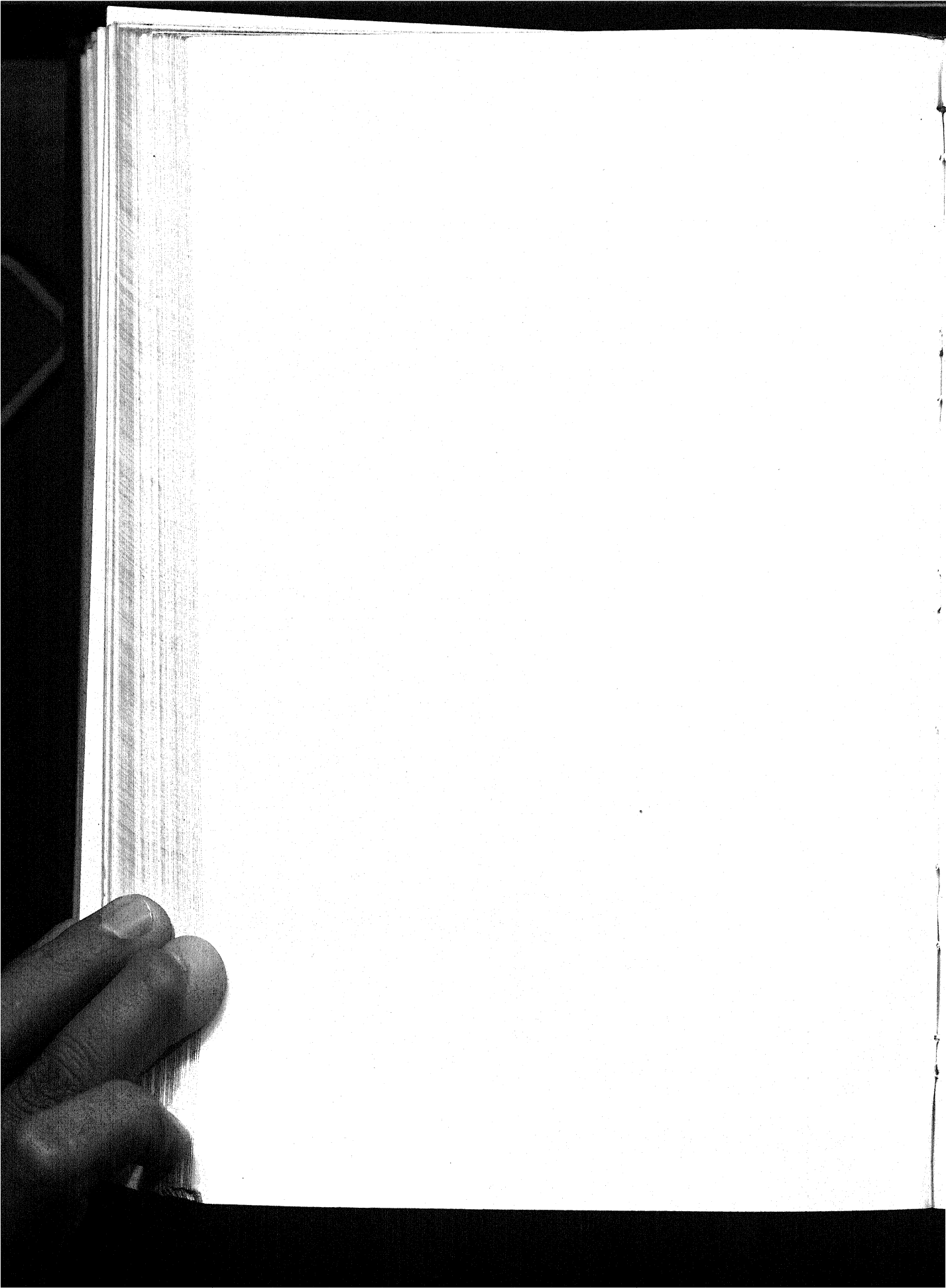
FOSTER: LEAF OF CARYA



FOSTER: LEAF OF CARYA



branching of the midrib obvious in its upper portion (× 2.5). FIG. 7, the spatulate-acute form and the venation of a seventh scale. Notice the characteristic trifurcation of the midrib near the apex of this organ (× 3). FIG. 8, the linear-rhomboid form and the venation of a seventh scale with a distinct, reticulately-veined, unevenly notched, terminal lobe (× 2.5). FIG. 9, the linear-rhomboid form and the venation of a seventh scale with highly developed reticulate venation in the terminal lobe. Note the weak branching of the single vein entering the apical pointlet (× 2.5). FIG. 10, the form and venation of the third cataphyll of a precociously expanding bud, May 13, 1930. This organ consists of a relatively simple-veined "petiolar" region and a small lamina in which there occurs a weak reticulate venation. Compare the mode of innervation of the laminar region of this organ with that of the terminal lobe of other cataphylls (*e.g.*, Pl. LXIII, figs. 4*b* and 5*b*) (× 6).



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ABSTRACTS OF THE PAPERS PRESENTED BEFORE THE PHYSIOLOGICAL SECTION OF THE BOTANICAL SOCIETY OF AMERICA, NEW ORLEANS, LA., DECEMBER 29–31, 1931 *

The Use of Dilute Nitric Acid on the Germination of Seeds of Poa Compressa. *Alice M. Andersen, Bureau of Plant Industry, Washington, D. C.*—Seeds of Canada bluegrass (*Poa compressa*) have been generally classed in the group of seeds requiring light for germination. For testing the germination of these seeds, they are sown in petri dishes on filter paper moistened with dilute (*N*/50) potassium nitrate solution and placed, with daily alternations, in the light at 30° C. for 6 hours and in the dark at 20° C. for 18 hours during a period of 28 days. Since the addition of dilute potassium nitrate solution is part of the procedure in the testing of these seeds, it seemed of interest to determine whether nitric acid would not act in the same manner as potassium nitrate in furthering germination. For this purpose, different concentrations of nitric acid were compared with *N*/50 potassium nitrate and water as stimulating agents both in the light and in the dark. Seeds sown in petri dishes on filter paper moistened with dilute concentrations of nitric acid varying from *N*/65 to *N*/1300 and placed, with daily alternations, in light at 30° C. for 6 hours and in the dark at 20° C. for 18 hours during a period of 28 days gave, through a range of *N*/520 to *N*/1040, 92 to 97 percent of germination equal to that obtained with *N*/50 potassium nitrate solution. Controls under similar conditions but with water gave 60 to 70 percent germination. In another series conducted in the same way but in the absence of light higher concentrations of nitric acid, *N*/195 to *N*/650, were necessary to give an equal percentage of germination (80 to 85 percent) to that obtained with *N*/50 potassium nitrate solution. The controls with water gave 20 to 30 percent germination.

Temperature Relations of CO_2 Production by Young Wheat Seedlings, As Influenced by Oxygen Pressure, Time and the Presence of Ethylene. *Burton E. Livingston and Warren B. Mack, Johns Hopkins University and Pennsylvania State College.*—This paper deals with mean hourly rates of CO_2 elimination from submerged wheat seedlings in an experiment period of 48 hours following a 42-hour germination period. The experiment period was divided into five observation intervals and no consistent influence of

* Printed and distributed in advance of the regular issue. Nov. 30, 1931.

time was apparent. Therefore the mean rates actually considered are for the last 36 hours of the period. A continuously flowing mixture of oxygen and nitrogen, or of these and ethylene, bubbled through the nutrient solution in which the seedlings were submerged. Oxygen pressures ranged from 0.6 to 98.3 percent, by volume; when ethylene was present its pressure was always 0.1 percent. Five different maintained temperatures were tested; namely, 10°, 15°, 20°, 25° and 30°. For each set of non-temperature conditions a temperature graph is available, with temperatures plotted as abscissas and CO_2 rates plotted as ordinates. The present report aims to call attention to notable differences in form among the twenty graphs that can be compared and to request suggestions concerning their interpretation. All graphs are of course concave upwards at the start and many of them maintain that form throughout, but those for two widely separated ranges of oxygen pressures are clearly convex upward in the region for temperature from 25° to 30°. The pressure ranges here referred to are approximately 9.8–16 percent and 90–98.3 percent. The point of inflection is lower on the thermometer scale for the lower pressure range than for the upper one. Further details may best be studied in connection with the graphs themselves.

Storage Tests with Seeds of the Wild Plum. *Johanna Giersbach and William Crocker, Boyce Thompson Institute for Plant Research, Yonkers, N. Y.—Prunus americana* Marsh. seeds were stored dry in the seed cabinet and in a heated cabinet adjacent to the chimney in the laboratory and also at 8° C. The germination tests made at intervals after storage were carried out at 5° C. which had been shown by previous experiment to be the optimum temperature. Up to the thirtieth month the seeds from the seed cabinet at room temperature storage showed no decrease in germination, while a gradual loss of vitality was obtained for the seeds stored in the heated cabinet with a complete loss after 50 months of storage. After 53 months the seeds stored in the seed cabinet at room temperature still germinated to the extent of 16 percent while the seeds from 8° C. gave 45 percent germination. Of the conditions tested for dry storage of seeds, 8° C. was found to be the optimum. When fresh seeds were dried over various concentrations of sulfuric acid and quick lime for 19 days, then planted, the germination percentage was slightly less than for seeds not artificially dried. Seeds dried in this manner were stored in sealed bottles for 53 months in the seed cabinet at room temperature, at 8° C. and at −15° C. Germination tests of these seeds showed no advantageous effects of artificial drying.

Germination of Seeds of the Silver Bell, Halesia carolina. *Johanna Giersbach and Lela V. Barton, Boyce Thompson Institute for Plant Research, Yonkers, N. Y.*—Plantings of fruits in mulched and board-covered cold frames gave good seedling production after one or two winters. Corre-

sponding plantings made in the greenhouse produced very few seedlings after two winters. Fruits planted in flats and allowed to remain in the greenhouse for three months after which they were transferred to mulched and board-covered cold frames always showed good germination after one winter outside. This points to the effectiveness of previous high temperature treatment in overcoming dormancy of *Halesia* seeds of certain crops at low temperatures. Lots of fruits were also stratified in moist granulated peat in electrically controlled ovens at constant temperatures of 1°, 5°, and 10° C. and at higher temperatures (15°, 20°, and 25° C.) from which they were transferred after one, two, and three months to 1°, and 5° C. Samples of fruits removed from stratification after one to six months at the various low temperatures and planted in soil in the greenhouse confirmed the results obtained from the outside plantings.

Germination of Seeds of the Bayberry. *Lela V. Barton, Boyce Thompson Institute for Plant Research, Yonkers, N. Y.*—Freshly harvested seeds of *Myrica carolinensis* Mill. gave fifty to ninety percent germination in eighteen to thirty days when they were planted in the greenhouse after stratification in moist granulated peat for three months at 1° or 5° C. This method was effective for both waxed and cleaned seeds, which still showed good germination after ten months of dry storage. Seeds planted in the greenhouse without previous cold treatment produced no seedlings in nine months.

The Wase Mutations of the Satsuma Oranges and their Temperature Requirements. *Walter T. Swingle, U. S. Department of Agriculture, Washington, D. C.*—The Satsuma orange, *Citrus nobilis*, Lour. var. *unshu*, is the chief commercial Citrus fruit in Japan. Branch mutations occur very infrequently giving rise to an early ripening mutation called Wase which in the Japanese climate with prolonged cool weather through the entire spring and early summer has a paradoxical combination of giant and dwarf characters, the buds, leaves and fruits usually larger than the mother variety, but the tree of slow growth and dwarfed. Some forty or fifty of these mutations have been found in Japan. Satsuma oranges have been grown in the United States for the last fifty years and are grown on a rather large scale from eastern Louisiana to northern Florida but so far no Wase mutations have arisen. The Wase mutations planted in the Gulf Coast region instead of remaining dwarfed, as in Japan, grow fully as fast as the mother variety making it clear that the dwarfed state of these mutations observed in Japan is due to an insufficiency of spring and summer heat, this being the outstanding difference in climate between the Gulf Coast region of the United States and the Citrus growing region in Japan. Preliminary tests made in the coast region of California characterized by prolonged cool weather in spring and low summer temperatures show a tendency to fruit very heavily and make slow growth as in Japan. Other

analogous examples of the effects of temperature on the origin and development of mutations are discussed by the author.

Freezing of Pollen: Evidence as to How Freezing Kills Plant Cells. *W. H. Chandler, University of California, Berkeley, Calif.*—Pollen was used because the environment of this cell can be controlled and because germination tests may give more dependable evidence of a living condition than microscopic examination of cells. The pollen was usually that of Lukens Honey peach, but that of Quetta nectarine, Ideal peach, Himalaya-berry, and the snapdragon also was used. The temperatures were usually − 15 to − 18° C. Dry pollen gave a high percentage of germination after many hours at these temperatures. Pollen moistened before being placed in the freezing room, or afterward, so that all the water was ice within 1.5 minutes, was practically all killed by a half hour at these temperatures. Less pollen was killed when, instead of distilled water, pure glycerine, concentrated sap from peach bark, or a concentrated solution of sucrose, glucose, or calcium chlorid, was put on the pollen in the freezing room. The concentrated sap and the glycerine were toxic, but much more so at 20° C. than at 0° C. or lower. Calcium chlorid was as toxic at 0° C. and at − 18° C. as at 20° C. The percentage germination was therefore not as great after freezing in calcium chlorid solution as after freezing in a concentrated solution of sugar, glycerine, or plant sap but was greater than after freezing in distilled water. These results are in harmony with those of Maximov with small sections of leaves and appear to support his view that freezing kills plant cells by the physical effect of the ice masses.

Freezing Point Depressions of Asparagus Shoots Determined by a Thermo-electric Method. *Evelyn I. Fernald, Rockford College, Rockford, Illinois.*—Freezing point depressions of actively growing shoots of asparagus were determined by a thermo-electric method. Copper-constantan thermo-couples were inserted one centimeter into portions cut from the asparagus shoots. The portions were usually two centimeters in length and one centimeter in diameter. The range of values tended to be high at the tip, decreasing to a point near the soil line where there was a slight rise, which was followed first by a decrease and then by an increase near the crown. The values for freezing point depressions tended to be highest in regions one centimeter and three centimeters from the tip of asparagus shoots still below the surface of the soil. A similar distribution of values was observed in the majority of shoots whose tips had emerged from the soil 1.5 to 3.7 cm. The distribution of the maximum and minimum values was less regular in shoots whose tips were more than 5 cm. above the soil. This work was done at the Boyce Thompson Institute.

The Disorganization of Cells of Mottled Orange Leaves. *H. S. Reed, University of California, Riverside, Calif.*—Mottle-leaf of citrus and

avocado has many similarities to the Rosette diseases of walnut and pecan. Leaf growth and structure are profoundly modified by the disease. The palisade cells of affected leaves are broad and frequently divided by transverse walls to form cubical cells. The protoplasm of palisade and mesophyll cells is so acid that neutral red is not able to penetrate. Neutral red, however, penetrates readily and accumulates in the vacuoles after a preliminary treatment with $N/50$ ammonium hydroxid. An acid dye, like eosin, kills the cells and enters freely. Methylene blue and other basic dyes penetrate rapidly. Nile Blue A is readily taken up by the cells and colors the fat droplets. The iron relations in healthy cells show no significant difference from those in mottled cells. The cells of the mottled leaves show varying stages of disorganization, terminating often in the complete disappearance of protoplasmic materials. The process of disorganization is accompanied by the accumulation of fatty substances. The chloroplasts at a relatively early stage are vacuolated then become fusiform and ultimately disappear. The vacuoles are filled with tannoid substances.

The Function of the Sieve Tube in Conduction. *A. S. Crafts, University of California, Davis, Calif.*—Granting that ringing and dissection experiments have proved the phloem to be the channel through which backward flow of assimilates takes place, and that movement is caused by pressure produced osmotically, the problem remains to find the actual conduits. Ruhland erroneously concluded that sieve tubes are no more permeable than parenchyma cells; Schmidt considered them not to be specialized conducting elements, while several workers find no evidence for pores, tubules or perforations in the end walls which would allow a mass flow. The phloem walls are highly hydrated, permeable, and occupy a considerable percentage of the total phloem volume. Yet if Poiseuille's formula is used to determine the pressures necessary to cause the required rates of flow through intermolecular spaces in the walls, the values found are too high, even when the formula is altered to apply to flow between parallel planes. Study of sieve tube ontogeny has shown that these elements have the properties of normal living cells while young. As they mature, however, they lose their nuclei, the protoplasm is altered and they apparently become completely permeable. If this condition can be shown to be general, it seems safe to conclude that sieve tube lumina provide the conduits through which a major portion of the organic nutrients flow in their movement from leaves to points of utilization. The author agrees with Kuhla, Schmidt, and others, that there is no evidence that perforations occur in the end walls of sieve tubes which would facilitate mass flow.

The Polarity of the Vacuole. *Silvestr Prat, Charles University, Prague, Czechoslovakia.*—The vacuole of the young growing cells of some marine species of *Cladophora* stains with neutral red much more intensively in the

apical part than in the basal part of the cell. The same is the case in the cells of *Codium bursa* and *C. adhaerens* with neutral red, brilliant cresyl blue, and vesuvin.

Metaxenia or the Direct Effect of the Pollen Parent on the Maternal Fruit Tissues and its Applications in Date Culture. *Walter T. Swingle, U. S. Department of Agriculture, Washington, D. C.*—The very striking direct effect of date pollen on size and time of ripening of the fruit was first announced at the February 1926 meeting of the American Association for the Advancement of Science. Roy W. Nixon published (Jour. Agr. Res., May 1, 1927; Jour. Heredity, June, 1928) accounts of his critically controlled pollination experiments giving the first indisputable proof of the direct effect of pollen on the maternal fruit tissues. In 1928 I discussed the implications of these newly discovered effects, proposing the name metaxenia for the distinct action of the male parent on the maternal tissues, and advanced a theory to account for these effects (Jour. Heredity, June 1928). L. M. Roh (1929, Horticultural Exp. Sta., Mleew, Russia) and B. R. Nebel (1930) published further on metaxenia in apples, and George J. Harrison (1931, Jour. Agr. Res.) published a striking demonstration of the occurrence of metaxenia in cotton. Experiments conducted at the U. S. Experiment Date Garden, Indio, California and in private date gardens of the Coachella Valley by Nixon have shown the possibility of utilizing metaxenia to great advantage in commercial date culture. The date blooming period lasts about six weeks in spring; the ripening of the fruit extends over a similar period in autumn The whole crop can be advanced or retarded in ripening by using suitable pollen. By using differential pollination the ripening season can be greatly prolonged or greatly contracted. All four of these applications of metaxenia are likely to prove valuable in date culture. They involve no extra cost to the grower since all date palms are pollinated artificially.

The Relation of Quantity to Stimulating Effect of Insulin, Glycerol, Urea, and Dipotassium Phosphate on Sugar Utilization by Spirogyra. *G. C. Wickwire, L. Kneer, and W. E. Burge, University of Illinois, Urbana, Ill.*—Large quantities of *Spirogyra porticalis* were collected and after removing excess of water by gently squeezing with the hands, portions of 75 grams were weighed out. Each of these batches was introduced into 300 cc. of 0.1 percent dextrose solution in glass dishes 18 cm. in diameter. Various amounts of insulin, glycerol, urea, and dipotassium phosphate were added and sugar determinations run according to the method of Benedict immediately and after 44 hours at the end of the experiments. In five series of experiments the average amount of sugar used by the controls was 32 percent. The *Spirogyra* in the sugar solution containing 0.5 percent glycerol used 33 percent of the sugar; that in the solution containing 2 percent glycerol used 40 percent; that in the solution containing

2.5 percent glycerol used 37 percent and the *Spirogyra* in the sugar solution containing 3 percent glycerol used 30 percent of the sugar. The *Spirogyra* in the sugar solution containing 0.005 percent dipotassium phosphate used 37 percent of the sugar; that in the solution containing 0.5 percent phosphate used 50 percent of the sugar and the *Spirogyra* in the sugar solution containing 2 percent phosphate used 55 percent of the sugar. The *Spirogyra* in the sugar solution containing 0.001 percent urea used 28 percent of the sugar; that in the sugar solution containing 0.005 percent urea used 31 percent of the sugar and that in the solution containing 0.1 percent urea used 30 percent of the sugar. *Spirogyra* in the sugar solutions containing 0.1 percent, 0.5 percent and 3 percent insulin used all of the sugar while that in the solutions containing 0.003 percent used 35 percent of the sugar. All of these experiments were carried out at ordinary room temperature and in diffuse light.

Some Effects of Ultra-violet Radiation upon Plant Enzyms. *Harry J. Fuller, Missouri Botanical Garden, St. Louis, Mo.*—It has been assumed that the injurious effect of ultra-violet radiation upon living tissue is due in part to the decomposition of enzyms, since ultra-violet radiation inactivates enzyms in vitro. The author has experimented with plant tissue badly injured by ultra-violet radiation in respect to catalase and diastase activity. It has been found that the activity of these enzyms actually increases to a considerable extent in tissues rayed with destructive wavelengths and that there is no evidence whatsoever of a diminution of enzym activity, a condition contrary to the results of previous investigators. Thus, it is demonstrated that the injurious effects of ultra-violet must be traceable to some physiologic derangement other than the inactivation of enzyms. It has also been shown that the inactivation of enzyms in vitro as a result of exposure to the radiations from a quartz mercury vapor arc are in considerable degree due to infra-red radiation.

Effects of Ultra-violet Radiation on the Calcium and Phosphorus of Plants. *F. Lyle Wynd and Harry J. Fuller, Missouri Botanical Garden, St. Louis, Mo.*—Tomato and cucumber plants which were stimulated to increased growth by ultra-violet radiation were analyzed for calcium and phosphorus in an attempt to determine whether or not a condition might obtain similar to that in animal tissue, in which calcium and phosphorus (chiefly the former) metabolism are increased by ultra-violet treatment. Analyses of these accelerated plants showed a decided increase in calcium content as compared with the control plants. Phosphorus, on the contrary, showed a decrease in the rayed plants, which does not correspond with the condition in animal tissue treated in a similar way. The calcium increase, however, is definite and significant.

The Effect of High Speeds of Rotation upon Certain Biological Phenomena. *Everett F. Davis, University of Virginia, Blacksburg, Va.*—

An apparatus which permits high speeds of rotation such as 3000 RPS has been used in certain physiological problems. The method consists essentially in both driving and supporting the rotor by means of a whirling film of air. The speed of rotation can be easily measured by means of an audio oscillator to a precision of about 0.1 percent. Certain data have been obtained as to the effect of such centrifugal forces as 1,000,000 + gravity upon the germination of seeds, protoplasm, and other biological phenomena.

Observations from the Microinjection of Acid-base and Oxidation-reduction Indicators into the Root-hair Cells of Trianea bogotensis, Karst. *Everett F. Davis, University of Virginia, Blacksburg, Va.*—In connection with further fundamental studies on the nature of the antagonism between the black walnut, *Juglans nigra* L., and numerous cultivated and native plants, an attempt was made to study the oxidation-reduction system, juglone-hydrojuglone, which naturally occurs in the walnut tree. It was thought that an explanation of resistance or lack of susceptibility to walnut toxicity might be associated with the reduction of toxic juglone to its non-toxic reductant, hydrojuglone. It therefore became of interest to determine the reducing intensity of the protoplasm of certain plants. This determination was first made on the root-hair cells of *Trianea bogotensis* Karst, which are peculiarly favorable for micrurgical methods. A series of acid-base as well as oxidation-reduction indicators were injected aerobically by means of the Chamber's manipulator. The pH of the cell interior was found to lie between 5.2 and 5.6. All of the oxidation-reduction indicators, including methylene blue, were reduced. The E_0 value or the E. M. F. of an equimolecular concentration of methylene blue and its reductant methylene white, is + .077 at pH 5.4. The E_0 value of an equimolecular alcoholic solution of juglone and its reductant, hydrojuglone, is + .142 at pH 4. It can be predicted from the data that juglone would be reduced if injected into the root-hair cells of *Trianea bogotensis*.

Color Development in Apples after Picking. *John M. Arthur, Boyce Thompson Institute for Plant Research, Yonkers, N. Y.*—The effects of a number of artificial light sources on the development of the red color of apples have been studied. Both the infra red and far ultra violet regions produce injuries on apples. The ideal light source for coloring apples is one which has little infra red or red but which has considerable ultra violet extending as far as the solar limit at wavelength 290 millimicrons. The mercury vapor lamp in uviol glass was found to be a good light source for this work when placed at sufficient distance from the fruit to avoid injury from either ultra violet or infra red. McIntosh apples were well colored by exposures of 48 hours to the rays from this lamp. Pieces of excised green peel floating upon water and exposed to the lamp developed color similarly. If these pieces were heated or immersed in alcohol so as to kill or injure the cells no color developed. Pigment production is believed to

be a function of the living cells. The time of exposure necessary for good color production was 40 hours for fruit gathered August 25. This period increases rapidly when fruit is either stored or left upon the tree. It is difficult to develop any color after November 15 even with prolonged exposures and no color was developed on fruit taken from storage in January. The spectral transmission of the red peel, green peel and extracted red pigment was determined.

Plant Growth under Shading Cloth. *John M. Arthur and W. D. Stewart, Boyce Thompson Institute for Plant Research, Yonkers, N. Y.*— During the years 1930 and 1931 sunflower, salvia, buckwheat, dahlia and tobacco plants were grown in the open and in each of 3 cages covered with shading cloth. Each cage was 50 feet by 12 feet and 7 feet high. All cages were ventilated by means of separate conoidal fans which maintained uniform temperature and humidity conditions throughout. The transmission of the various cages as measured on May 28 was 100, 78, 58 and 35 percent of the total solar radiation. The green and dry weights, percentage moisture and height of the plants was determined. All plants except salvia grown during the months of June and July produced greater dry and wet weight of tissue when shaded as compared with open sunlight. Tobacco reached a maximum dry weight under muslin cloth at 35 percent of outside intensity. Sunflower produced at a maximum when only slightly shaded by hospital gauze at 78 percent transmission. Dahlia and buckwheat reached a maximum under cheesecloth at 58 percent transmission. Maximum dry weight production of the same species of plants grown during August and September at a lower light intensity and shorter daylength moved up into cages with higher transmission values. Sunflower, tobacco and buckwheat were all highest in open sunlight. Light intensity is apparently too high for maximum dry weight production during June and July for a number of plants but is optimum or barely sufficient as the season progresses in August and September.



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